GENETICS

A periodical record of investigations bearing on heredity and variation

Founded in 1916 by GEORGE H. SHULL

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VOLUME 41 SEPTEMBER, 1956 NUMBER 5

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PUBLISHED BIMONTHLY AT BALTIMORE, MARYLAND
BY GENETICS, INC.

(Date of issue, November 5, 1956)

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Volume 41 SEPTEMBER, 1956 Number 5

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January–November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Mount Royal and Guilford Avenues, Baltimore 2, Maryland.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

Entered as second-class matter at the postoffice at Baltimore, Md., under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

ABSTRACTS OF PAPERS PRESENTED AT THE 1956 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

STORRS, CONNECTICUT, AUGUST 27 to 29, 1956

ABRAHAMSON, SEYMOUR, Indiana University, Bloomington, Ind.: *The effects on rearrangement frequency of different oxygen tensions either during or between fractionated X-ray treatments of Drosophila oocytes.*—The work of HERSKOWITZ and ABRAHAMSON (1955) demonstrating that half-translocation frequency was X-ray-intensity dependent made it feasible to test separately the effects of oxygen tension on the breakage and restitution processes in experiments on *D. melanogaster* similar to those performed by WOLFF and ATWOOD (1954) and WOLFF and LUIPPOLD (1955) on *Vicia faba*. Virgin females carrying the "snoc" attached-X and marked Y (sc^{v1}. Y^{8}) chromosomes, 3–5 days old, were irradiated in lucite chambers and mated to *y* sc^{8l} *B In49 v*/Y males. F_1 flies from eggs laid within 4 days after treatment were scored for detachments of attached-X chromosomes. The total dose, 2000r, was delivered in 70 seconds, either continuously or discontinuously, in two 35-second periods, 58 minutes 50 seconds apart. With irradiation in air the continuous treatment gave 4.2 ± 0.3% detachments, the discontinuous, simultaneous treatments separated by an interval in N_2 or O_2 respectively gave 4.1 ± 0.4% and 1.9 ± 0.2%. Thus without O_2 the ends appear to remain open but with O_2 they unite. With simultaneous, discontinuous irradiations in N_2 and O_2 respectively, separated by an interval in air, the frequencies were 1.1 ± 0.1% and 2.5 ± 0.3%. This indicates that oxygen promotes breakage. These results are similar to WOLFF and co-workers' in demonstrating two contrary influences of oxygen in the production of chromosome rearrangements. (This work has been supported by a grant to DR. H. J. MULLER and associates from the U. S. Atomic Energy Commission (Contract AT(11-1)-195), and by the Indiana University Eigenmann Fellowship in Zoology.)

AGUIRRE, F., and N. S. SCRIMSHAW, Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.: *Ability of representative groups of Guatemalan Indians to taste phenylthiourea.*—With the assistance of the Instituto Indigenista 385 adults of Mayan Indian origin in 22 widely scattered localities in Guatemala were tested for ability to taste phenylthiourea at concentrations of 1:100,000, 1:40,000 and 1:8,000. One to two drops of the most dilute solution were placed on the tongue. If no taste was reported the mouth was rinsed with water and the next dilution tested in the same manner. In combined results of tests at the three concentrations, 332 persons stated the taste to be bitter, 32 acid, 16 sweet, 2 salty and only 3 (0.8%) were unable to taste the compound. At the 1:100,000 concentration 38.4% of the total group was able to taste it. At 1:40,000 an additional 47.3% could do so, and 13.5% did not detect the taste until a concentration of 1:8,000 was employed. It would appear that surveys employing phenylthiourea concentrations of less than 1:8,000 will miss a significant proportion of tasters. In comparison with European populations the percentage of non-tasters in the Indian groups was exceedingly low, but similar to that reported for African Negroes. Of 44 non-Indian persons living in Guatemala City, 9 were able to detect the phenylthiourea at 1:100,000, 15 at 1:40,000, 12 at 1:8,000 and 8 (18.2%) were unable to taste it.

ALEXANDER, MARY L. The Genetics Foundation, University of Texas, Austin, Texas and Biology Division, Brookhaven National Laboratory, Upton, N.Y.: *Dominant lethal and translocation damage in the immature germ cells of Drosophila virilis from fast neutrons.*—The postmeiotic germ cells of *Drosophila virilis* show an increase in translocation damage which follows a "one-hit" curve and increases in proportion to the increase in the dose of fast neutrons. The damage in the more mature cells (A & B) was 2.47% translocations at the lowest dose (estimated dose of 65 rep) and increased to 5.95% when the dose was doubled (130 rep) and 13.78% for 260 rep. The most sensitive cells recovered were from mating period D and gave values of 6.03% translocations for the lowest dose, 8.29% for the second and 20.00% for the highest dose. The intermediate period (C) gave values which were intermediate between these two types. The most sensitive cells show 2.4 times more damage than the less sensitive cells of period A and B at the 65 rep dose level. No translocations were recovered from

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the premeiotic cells (periods F, G, H). The dominant lethal damage was measured throughout the cycle in both pre- and postmeiotic cells. In the postmeiotic cells, the highest peak of damage measured as dominant lethals coincided with the high point of translocation damage and the dominant lethal damage was increased about 1.5 to 2 times in the most sensitive cells at the 65 rep dose level. The damage in premeiotic cells dropped to a value of 14 to 17% as compared to 40% for the most sensitive cells recovered from mating periods D and E. Small egg samples were checked for the presence of sperm in some mating periods to distinguish between cell degeneration, as expressed as an absence of mature sperm, and genetic lethality. None of the eggs checked in period G contained sperm thus showing the absence of available sperm in this period. Neither the previous period (F) nor following period (H) showed an indication of this type damage. (Fast neutron treatment was carried out at Brookhaven National Laboratory, Upton, L. I., N. Y. The author's present address is Biology Department, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston 25, Texas.

ALLEN, SALLY LYMAN, University of Michigan, Ann Arbor, Mich.: *Stabilization of unstable nuclei in Tetrahymena pyriformis.*—The control of mating types in *T. pyriformis*, variety 1, has been shown to be under the influence of the macronucleus. Mating type differentiation involves the progressive elimination of nuclear potentialities during maturation until usually only one remains. Occasionally this process may extend into maturity with the result that mating type differentiation is incomplete. This may be detected by the observation of mating among the descendants of a single vegetative cell (selfing clones). Such a nuclear condition is unstable since clones derived from selfers may complete their differentiation to a single mating type, and selfing is no longer observed. However, the unstable nuclear condition may be perpetuated by selection for over 1000 cell divisions. Analysis of selfing clones shows that they contain at least two mating type potentialities in certain combinations with types I and VI in most frequent association. Furthermore, the unstable nuclear condition is highly heritable, and among cells with a recent common origin stabilization to only one of the two types occurs. Starvation forces an increase in stabilization to the one type. Studies of the time course of stabilization in subclones of a selfer show a wide range in rates of stabilization and an inverse correlation between the rates to the two possible mating types. The implications of these results for interpretations of nuclear differentiation will be discussed. (Work supported by grants to D. L. NANNEY from the National Science Foundation).

ALTENBURG, LUOLIN S., The Rice Institute, Houston, Texas: *The production of mutations in Drosophila by ethyl formate vapor.*—The polar caps of dechorionated fertilized eggs of Drosophila containing the cells of the early germ track were exposed for from 5 to 20 minutes to the vapor of ethyl formate in a moist sealed chamber. After the eggs hatched, the males were tested by the "sifter" technique for the presence of lethal mutations in the second pair of autosomes. The mutation rate was found to be $4.7 \pm 1.6\%$ as contrasted to a control rate of $0.5 \pm 0.3\%$, the induced mutation rate being $4.2 \pm 1.6\%$. Treatment of the eggs was accompanied by a very high mortality, only 7.5% of the total number of treated eggs developing into fertile males. (This work was supported by a grant received for work of EDGAR ALTENBURG and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

ALTENBURG, LUOLIN S., The Rice Institute, Houston, Texas: *The effect of intensity on the mutagenic activity of ultraviolet light when administered in relatively low doses to Drosophila.*—The frequency of lethal mutations induced by relatively low doses of "germicidal" ultraviolet (chiefly 2537 A) in the second chromosomes of the embryonic polar cap cells of Drosophila depends in part on the intensity of the incident light. It has been found that a dose of about 80 ergs/mm^2 applied for 1½ minutes at a distance of 180 cm from the lamp produces a mutation rate of $4.8 \pm 1.1\%$, whereas the same dose (as measured by a General Electric germicidal meter) applied for 6 minutes at a distance of 300 cm yields a mutation rate of only $1.0 \pm 1.0\%$. Moreover (in an earlier series of experiments), when a dose of 100 ergs/mm^2 was administered by irradiating the polar caps for 2 minutes at a distance of 180 cm from the source, a mutation rate of $5.9 \pm 1.7\%$ was recovered, as contrasted to a rate of $1.4 \pm 0.5\%$ when the same dose was given with irradiation carried on for 8 minutes at a distance of 360 cm

from the source. Previous work had shown that at dosage levels at least five times those used in the present experiments, the mutation rate induced by ultraviolet was independent of the intensity over a nineteen-fold range in intensity, when the total dose was held constant. (This work has been supported by a grant received for work of EDGAR ALTENBURG and associates from the National Institutes of Health, United States Public Health Service.)

ALTENBURG, LUOLIN S., and EDGAR ALTENBURG, The Rice Institute, Houston, Texas: *The synergism between ultraviolet light and 8-methoxy psoralen in their mulagenic effectiveness in Drosophila.*—The frequency of lethal mutations in the second chromosomes of germ cells in the embryonic polar cap is not increased by immersing the polar caps in a 0.05 M solution of 8-methoxy psoralen in water and alcohol during the polar cap stage (the mutation rate being $0.3 \pm 0.2\%$). If a low dose of ultraviolet light of 2537 A wavelength (capable of producing a mutation rate of $0.8 \pm 0.6\%$) is applied to the polar cap cells by irradiating them through the psoralen-containing solution of about 2 mm thickness, the mutation rate is increased to $1.7 \pm 0.8\%$, but because of the rather large error due to the clustering of mutants of common origin inherent in this technique, this increase may not be significant. However, if the psoralen solution is poured off the eggs after a pre-soaking period of 25 minutes and the polar caps immediately irradiated with the low ultraviolet dose, a mutation rate of $3.8 \pm 1.5\%$ is obtained, a rate which is significantly different from that obtained with the ultraviolet alone, indicating a synergistic action between the two agents.—Possibly the lower rate obtained when radiation is administered at the same time as the psoralen, rather than as a posttreatment, is due to the fact that the ultraviolet passes through a layer of water (used as a solvent for the psoralen) of about 2 mm thickness. Results of recent experiments (unpublished) indicate that 2 mm of water when covering the polar caps significantly reduces the mutation rate obtained by a dose of ultraviolet four times that used in these experiments as compared with the rate produced by a similar dose of ultraviolet which strikes the eggs without first passing through a layer of water ($0.9 \pm 0.5\%$ versus $6.7 \pm 2.5\%$). In all these experiments the polar cap ends of the eggs were inserted into holes in aluminum foil. The foil may possibly be involved in the relative rates obtained rather than the water alone, since a layer of water of 2 mm thickness absorbs very little ultraviolet of 2537 A wavelength. (This work was supported by a grant received from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.).

ANDERSON, LULU F., and DALE STEFFENSEN, Biology Department, Brookhaven National Laboratory, Upton, N. Y.: *Tube nucleus divisions in Tradescantia pollen associated with a translocation.*—During a series of mineral deficiency studies on *Tradescantia paludosa* (clone 5 of SAX), a seedling mutant was detected by cytological screening. This plant showed an almost constant rate of 25 per cent pollen abortion. Smears of meiosis showed the abortion to be due to the segregation of a translocation which had the typical ring of four at the first meiotic metaphase. On further examination of the pollen, it was observed that the tube nucleus, which normally degenerates, sometimes underwent a complete division. This resulted in pollen with three nuclei, the dark-staining generative and two lighter staining nuclei of similar size. All stages of division in the tube nucleus have been observed. Although trinucleate cells were always present in pollen samples of the mutant plant, their frequency showed a large variability probably due to environmental factors.—The plant was selfed in order to determine whether the tube nucleus phenomenon was associated with the rearranged chromosomes. The three types expected if the two were directly related would be (1) the normal, (2) the heterozygous with tube nucleus divisions and abortion, and (3) the homozygous rearranged with tube nucleus divisions and no abortion. Examination of the progeny of the selfing showed a fit to a 1:2:1 ratio by the chi square test of normal, heterozygous, and modified types, respectively. The ability of the tube nucleus to undergo division, therefore, seems to be a result of the chromosome rearrangement. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

ATKINSON, G. F., J. G. ROSS, and C. J. FRANZKE, South Dakota State College, Brookings, South Dakota: *Differential reaction of two varieties of sorghum to colchicine treatment.*—Sixty seedlings of

each of two varieties of sorghum, Experimental 3 and Norghum, were treated with colchicine. Forty-three Experimental 3 and fifty-four Norghum plants survived to maturity and were compared with untreated checks of each variety. In Experimental 3, treated plants were significantly more variable than untreated plants for the characters presence of awns, plant height and number of tillers. Eighteen of the treated plants were distinct mutants for one or more characters. All Experimental 3 plants were diploid. In Norghum, treated plants were no more variable than untreated plants, and no distinct mutants were found. Three treated Norghum plants, out of thirty examined, were found to be tetraploid while no tetraploids were found in the untreated plants.—Examination of chromosome behavior in colchicine-treated root tips and cytological and histological examination of the c-tumors of colchicine-treated seedlings of the two varieties revealed no basis for this difference in reaction.

BALBINDER, ELIAS, Indiana University, Bloomington, Ind.: *Two loci controlling the maintenance and stability of the cytoplasmic factor "kappa" in stock 51, var. 4 killers of Paramecium aurelia.*—When stock 51 killers are crossed to stock 29 sensitives in var. 4 of *P. aurelia* and an F_2 by autogamy is obtained from the killer parent, a 1:1 ratio of killers:sensitives is obtained from the segregation of the alleles *K* and *k*. However, about 50 to 70% of the segregating killer clones are characterized by the production of killer as well as sensitive lines of descent. Clones of this type are designated as "mixed". SONNEBORN (1947) reported a gene, *S*, present in stock 29 and not linked to *k*, associated with the production of mixed clones. Its allele, *s*, occurs in the killer stock 51. Further investigation has disclosed the presence of two independently segregating stock 29 genes, S_1 and S_2, which affect the stability of the cytoplasmic factor kappa when introduced into the genome of stock 51 killers by selection and backcrossing. In killers of the constitution $KKS_1S_1S_2S_2$, kappa will disappear completely from the clone at 27°C under most conditions of culture. The time for total loss varies, but has never been found to be below 70 fissions. Killer clones of the constitution $KKs_1s_1S_2S_2$ or $KKS_1S_1s_2s_2$ do not lose kappa entirely at 27°, although sensitives are sporadically produced by such clones in variable numbers. The allelic relations of S_1 and S_2 to SONNEBORN's gene *S* are under investigation. The mechanism involved in the elimination of kappa from animals of mixed clones appears to be very complex. (Work supported by Atomic Energy Commission grant to T. M. SONNEBORN.)

BAYLOR, MARTHA BARNES, and D. D. HURST, University of Michigan, Ann Arbor, Mich.: *Genetic suppression of host range in the T_2 coliphage.*—The host range of the coliphage, T_2, has previously been shown by us to be under the control of a series of loci acting as a polygenic system, the members of which quantitatively and additively increase host range. Thirteen such loci, designated as ht_1–ht_{13}, have been mapped on the T_2 linkage groups. There is now evidence which shows that host range is also under the control of another series of loci, *su*, each of which reduces the host range. The measurement of the number of mutants of decreased host range in populations of parents containing various of the *ht* markers indicates a high mutation rate which is best interpreted as a cumulative mutation rate of many loci. The three suppressor mutants so far tested by suitable genetic crosses are demonstrated to involve loci distinct from one another and distinct from the mapped *ht* loci. These mutations seem to be able to reduce the host range regardless of which of the *ht* markers are within the genome. One interpretation of these results is that the suppressor condition actively suppresses the formation of a substance necessary for the expression of host range, while a second is that the *ht* and *su* states are allelic forms of the same loci, the final phenotype being the resultant of the relative numbers of the loci in the two different states. (This work was supported by grants from the National Institutes of Health, U.S.Public Health Service and from the Smith, Kline and French Foundation.)

BIANCHI, A., Botanical Museum of Harvard University, Cambridge, Mass.: *Defective endosperm mutants from maize-teosinte derivatives.*—At least 28 defective endosperm mutants have been recorded in maize-teosinte derivatives. To indicate their origin, they are designated as de^{n1}, de^{n2}, etc.. Data so far obtained suggest that the majority of the mutants are genetically different.—One of the com-

mon features of these mutants is a characteristic heterogeneity in their segregations. The total number of recessives on the segregating ears may, on the average, approach the expected monomendelian percents, but individual ears vary greatly.—This striking heterogeneity may be due to one or more of the following causes. The mutant genes are themselves mutable and highly unstable. This is known to be true of de^{f5}, which MANGELSDORF is studying extensively; but de^{f4}, de^{f17} and de^{f19} also do not breed true. Genetic background may result in low ratios: de^{f1}, for example, segregates poorly in the selfed strains, but gives good Mendelian ratios in hybrids. Differential fertilization, due either to gametophyte factors or to de^{t} genes themselves, may be involved. In the case of de^{f3} and de^{f9}, the upper part of the ear often shows higher frequencies of defectives than the lower.—Weights, expressed in percent of the normal sib seeds, range from 2–8% for de^{f12}, de^{f21}, de^{f13} to 62–72% for de^{f10}, de^{f20}. Germination varies from 0–1% for the more defective mutants to 91–98% for de^{f14} and de^{f19}. Germination does not seem to be a simple function of the weight of the defective kernels; also, there is an interaction between some of the defective mutants and su_1.—Linkage relationships between most of the mutants and marker genes for chromosomes 2, 4, 5, 6, 7 and 9 have been tested. Mutants de^{f1}, de^{f2}, de^{f3}, de^{f20} and de^{f22} have been located on chromosome 4. Genes de^{f1} and de^{f2} are closely linked, and when crossed give rise to a good example of balanced lethals. Mutants de^{f2} and de^{f3} seem to be allelic. The gene de^{f11} is linked with lg_1, de^{f23} with gl_1, de^{f26} with *pr* and de^{f28} with y_1.—Cytological studies do not reveal any chromosome aberrations regularly associated with the defective mutants.

BLUMEL, JOHANNA, Department of Surgery, Orthopedic Division, University of Texas, Medical Branch, Galveston, Texas: *Paper chromatographic and colorimetric analysis of salivary glands of certain diptera.*—Salivary glands of four species of Drosophila and one member of the Phoridae were analysed for their amino acid, carbohydrate, and pentose content. By means of the two-dimensional partition paper chromatography technique and colorimetric tests employed quantitative and qualitative differences were demonstrated. The following amino acids were demonstrated in the salivary glands of virilis, repleta, texana, melanogaster and Phorid: proline, leucine, valine, alanine, glycine, glutamic acid, aspartic acid, arginine, lysine, serine, threonine, tyrosine, and phenylalanine. In addition to these, gamma aminobutyric acid, possibly taurine, and ethanolamine were demonstrated in Phorid. Taurine was also present in melanogaster, repleta and virilis. Ethanolamine was found to be present in repleta, texana and virilis. Two other compounds were present in virilis, X_1 a completely unidentified compound which gives the ninhydrin reaction, and cystine or a derivative of cystine.—Colorimetric tests employed to determine the carbohydrate and pentose content gave the following results: In 100 salivary glands of virilis analysed, 10 percent of 454 gamma of total carbohydrate was in the form of pentose. In texana 12 percent of the 357 gamma carbohydrate constituted the pentose fraction. The other two species of Drosophila showed a much higher percentage of pentose. *D. melanogaster* with a total carbohydrate content of 163 gamma showed that 22 percent was in the pentose form. *D. repleta* with 241 gamma carbohydrate had a 20 percent pentose content. Phorid, a non-Drosophila, had 22 percent of its total carbohydrate in the form of pentose.

BRILES, C. O., University of Wisconsin, Madison, Wis.: *Two loci affecting the cellular antigens of the chicken.*—Immunogenetic studies were carried out on the antigens of erythrocytes in chickens of testcross progeny. The antigens were identified by reagents which contained antibodies prepared from iso-immune sera, usually by differential absorption with erythrocytes of selected individuals. Testcross data indicated that each antigenic substance segregated in the offspring of each particular family as if it were the product of a single gene. These substances appear to belong to two genetic systems, A and E. The distribution of the antigenic substances among these testcross progeny provided immuno-genetic information of the causative genes. For example, five reagents, A6, A7, A8, and A68, reacted with eight cellular antigens of the A system as follows: A_6, A_7, A_8, A_9, A_{68}, A_{78}, A_{79}, and A_{689}. The data indicated that each of the eight antigens is determined by a different *A* allele. In addition a ninth allele, *a*, is proposed because some cells, presumably from *aa* birds, fail to react with the above reagents of the A system. In a similar manner using reagents E1, E12 and

E24, six alleles were recognized at the E locus; E^1, E^2, E^{12}, E^4, E^{24}, and *e*. Suggestive evidence that the A and E loci are closely linked will be discussed. (The reagents detecting the A_7, A_9, E_1 and E_2 antigens were duplicates of reagents previously prepared by W. E. Briles at the Texas Station.)

BRINGHURST, R. S., University of California, Davis, California.: *Colchicine induced polyploidy in the avocado.*—Persistent tetraploid tissue has been induced in the Fuerte avocado clone and in seedlings of the Mexicola variety by the colchicine technique. Typical gigas characteristics were evident in successfully treated plants, including broad, thick leaves and large stomata, flowers, and pollen. some plants were chimeral, with haploid and diploid pollen grains forming in the same locule of certain anthers. The tetraploid nature of the plants was confirmed by cytological examination. The diploid pollen germinated as rapidly as the haploid, and the pollen tubes penetrated as readily. No fruit set was obtained with tetraploid Fuerte pollen, but this is not significant since the diploid Fuerte is very sporadic in bearing. The tetraploids appeared to be less hardy to cold than comparable diploids in all cases.

BRINK, R. ALEXANDER, University of Wisconsin, Madison, Wis.: *A regularly reversible change in determinative action at the R locus in maize.*—*R* male gametes from *RR* (self-colored aleurone) plants in an inbred line produce a darkly mottled aleurone in testcrosses with *rr* (colorless aleurone); all the *R* male gametes from RR^{st} (heterozygous stippled) sibs, on the other hand, give weakly colored, or sometimes colorless, aleurone in the same testcross. R^{st} male gametes from $R^{st}R^{st}$ and RR^{st} individuals in testcrosses with *rr* are alike in effect on endosperm phenotype. *RR* progeny of selfed RR^{st} heterozygotes regularly show the darkly mottled phenotype in testcrosses with *rr*. The modified form of *R* from RR^{st} plants persists for at least one generation in *Rr* plants from *rr* ♀ × RR^{st} ♂ crosses, and thus is propagated in the sporophyte as well as in the gametophytes and endosperm. An *R* which has regained normal pigment-producing action in a derived *RR* plant, after having been altered in determinative action in an RR^{st} heterozygote, retains its normal action in *Rr* testcross progeny, and appears to be stable. No unusual properties of the *R* allele in question have been observed except in association with R^{st}.

BUTLER, L., and L. O. CHANG, University of Toronto, Toronto, Ontario and Ontario Agricultural College, Guelph, Ontario: *Graft-relationships of the anthocyanin mutants of the tomato.*—The green stem mutants *a*, *al*, *ag* and *aw* show slight phenotypic differences and are genetically distinct, belonging to different chromosome groups. Each mutant was grafted onto the purple-stemmed wild type, and reciprocal grafts were also made using mutant stock and wild type scion. In each case the stem color stopped at the graft junction, showing the cell specificity of the anthocyanin and the intermediate products. Each mutant type was also grafted reciprocally onto the other mutant types, but no purple showed at the graft junctions. Paper chromatography was used to detect chemical differences between the green-stem mutants. The chromatograms showed that both purple and green-stem types had four spots, and the anthocyanidin present was tentatively identified as pelargonidin. While the number of spots was the same in all mutant plants and their normal alleles, the R_F values for some of the spots were different and offer a possible chemical method of identifying the mutants. When the plants were grown under conditions of phosphate deficiency the leaves and stems of the wild type became intensely purple, whereas the stems of all mutant types remained green. The leaves of *a*, *al* and *aw* remained green, but the leaves of *ag* turned purple.

CARSON, HAMPTON L., Washington University, St. Louis, Missouri: *Response of Drosophila robusta to selection for motility.*—Measurement of the widely used polygenic characters, such as bristle number, is so laborious as to preclude controlled selection experiments on replicate wild strains, as in population analyses. The character of motility towards light is suitable for such studies. Flies are reared in uncrowded cultures with uniform light and temperature; 100 ♀♀ and 100 ♂♂ in two groups of 50 each, are aged uniformly (♀♀—7 days; ♂♂—9 days). Fifty flies to be tested (always between 9 and 10 A.M.) are shaken into a clean, empty ½ pt culture bottle. A second empty bottle is inverted over the mouth of the first and the two held in line. The second bottle is held away from

the operator, at an angle slightly above the horizontal, 10 cm from a 60 watt bulb. After a 15 sec interval, the first bottle, containing "non-movers" is set aside and the "movers", in the second bottle, are immediately given a second trial. "Movers" are given repeated trials until only two flies will transfer. These comprise two of the eight parents selected for the next generation. Group performance is measured by the number of fly-moves observed during the test. Controls, in which parents are chosen at random, are simultaneously tested. Of 25 wild strains selected for six generations, 18 have responded; in some cases the response is great. The method has promise as a means of assay of the genetic variability of populations and the speed with which this variability may be released.

CHOVNICK, A., R. J. LEFKOWITZ, and DOROTHY R. MCQUINN, Department of Zoology, University of Connecticut, Storrs, Connecticut: *Complexity at the garnet locus in Drosophila melanogaster.*—An investigation into the possibility of pseudoallelism at the garnet locus (chromosome 1-44.4) of *Drosophila melanogaster* is in progress. Examination is made of backcross offspring produced by females heterozygous for two mutant alleles of garnet, and heterozygous for closely linked markers on either side of garnet (sable and pleated). Such females are also heterozygous for the third chromosome inversion, *In(3R)Mo, Sb, sr.* —Three series of crosses have been studied at this time. (1) 43,895 X-chromosomes segregating from females of genotype g/g^2 have been assayed, and all carried a mutant garnet allele. (2) 17,937 X-chromosome segregating from females of genotype g^2/g^3 were assayed. Among these, one g^+ X-chromosome was found. From the linked markers present, the origin of this chromosome is inferred to have arisen from a crossover event suggesting pseudoallelism. (3) 43,749 X-chromosomes segregating from females of genotype $g^2/g^{ao}e$ were assayed. Among these, two g^+ X-chromosomes were recovered in which the linked markers, present in both cases, supported the view that these chromosomes arose from crossovers between pseudoalleles. A third g^+ X-chromosome arose which did not exhibit recombination for the closely linked markers. Several possible alternatives for the origin of this chromosome are under investigation. It has not been possible to recover double mutant chromosomes from these crosses. An attempt to recover such double mutants from crossovers in heterozygous attached-X females is in progress. While these data clearly indicate complexity at the garnet locus, it would be premature to specify pseudoallelic relationships.

CHU, ERNEST H. Y., and NORMAN H. GILES, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *Primate chromosome complements determined by tissue culture techniques.*—The chromosome numbers and karyological characteristics of six species of monkeys have been determined in tissue cultures of kidney epithelial cells (kindly furnished by DRS. J. L. MELNICK and G. D. HSIUNG of the Yale Medical School). For cytological studies, two types of materials were used: (1) cell suspensions obtained by removing cells from the culture vessel walls by incubation with trypsin or versene solutions and (2) cells proliferating on cover slips in depression (LEIGHTON) tubes. In both procedures cells were pretreated with hypotonic saline to spread chromosomes (HSU and POMERAT) and smear-stained with acetocarmine. The chromosome numbers of the materials studied appeared to be essentially constant with very occasional polyploid cells. Chromosome counts were determined for the following species, all Old World monkeys in the family Cercopithecidae: (1) *Macaca mulatta* (Rhesus macaque, ♂ and ♀)—42; (2) *Papio papio* (Guinea baboon, ♂)—42; (3) *Papio doguera* (olive baboon)—42; (4) *Erythrocebus patas* (military red grass monkey, ♀)—54; (5) *Cercopithecus aethiops*, subspecies *sabaeous* (African green monkey, ♂) and subspecies *tantalus* (African white monkey, ♂ and ♀)—60; (6) *Cercopithecus nictitans* subspecies *buttikoleri* (white-nose or spotted-nose monkey, ♀)—66. —The fact that all somatic chromosome numbers in primates determined to date, including that of man, are multiples of six appears noteworthy, although the interpretation of this situation is not yet clear. Photomicrographs will be presented to illustrate details of chromosome morphology and number in these species. (This research has been supported in part by a grant from the Atomic Energy Commission).

CLARK, KARIN H., McGill University, Montreal, Que., Canada: *Histological investigation of cortisone induced cleft palate in mice.*—Cortisone acetate given to pregnant mice has been shown to produce cleft palates (without cleft lips) in the offspring. The A/Jax strain is highly susceptible to this treat-

ment, while C57BL is relatively resistant. The difference in susceptibility has been shown to be polygenically inherited.—Histological studies of the embryonic palate show that there is in the palatine shelves a material which stains well with aldehyde-fuchsin (Gomori), and which increases in concentration with advancing stages of palate closure. The stainable elements are presumed to be elastic fibers, although their fibrous nature is not very marked. Untreated A/Jax embryos have a lower concentration of these elements than untreated C57BL embryos. Both types of treated animals show less stainable material than the normal embryos, but the concentration in the treated A/Jax is less than in the treated C57BL animals. It is suggested therefore that the stainable material may be the physical basis for the force which enables the palatine shelves to slide over the tongue in order to meet and fuse in the midline. According to this theory cortisone treatment causes cleft palate by interfering with the formation of this material. Thus the genetic constitution of the C57BL strain determines that the normal concentration of these elements is well above the threshold necessary for palate closure. Consequently cortisone treatment causes only a few of these embryos to fall below this threshold, while in the A/Jax animals, where the normal concentration is less, a high frequency of cleft palates results.

CLAYTON, FRANCES E., Department of Zoology, University of Arkansas, Fayetteville, Arkansas: *Eye pigmentation in the lozenge allelic series of Drosophila melanogaster.* The effects produced by ten members of the lozenge series of mutants in *Drosophila melanogaster* on the quantity and distribution of eye pigments in the cells of the ommatidia have been analyzed histologically. Pigment distribution in the wild type eye, brown, scarlet and vermilion were analyzed for comparison of normal pigmentation with the irregularities in the lozenge eyes. When both red and brown pigments are present the lozenge pseudoalleles may be classified into two groups which correspond in general with the classification based on structural abnormalities of the ommatidia. The first group consists of those mutants which range from normal coloration to a color darker than wild type: lz^{37}, lz, lz^{BS}, lz^{9}, lz^{34}, and lz^{v4}. The second group is composed of those mutants with a brownish eye color: lz^{3}, lz^{s}, lz^{sB} and lz^{36}. When the brown pigment is analyzed separately a single series may be arranged, with the exception of lz, in the order of decreasing amounts of brown pigment. This seriation corresponds to a series based upon increasing severity of ommatidial abnormalities. Comparison of the lozenge effect with *st* and *v* reveals variations in the amount of pigment in the pigment cells although the general distribution is similar. Abnormalities in the arrangement and structure of the pigment cells, together with degeneration of cells in the severe alleles, can account for variations in the distribution of pigment and some quantitative changes but other factors seem to be involved. Alleles which cannot be distinguished on a structural basis may vary considerably in the amount of pigment present in the ommatidial cells.

COHEN, CARL, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Blood groups in the rabbit. The measurement of gene dosage effects using isoimmune sera.*—Of the five loci which control the occurrence of eight rabbit erythrocytic antigens which we have detected so far, one locus, having three alleles (Hg^A, Hg^D, and Hg^F), each of which give rise to a detectable antigen (antigens A, D and F respectively), lends itself to the study of gene dosage in relation to blood groups. It has long been known that there may be more antigen in blood homozygous for a particular gene than in the blood of the heterozygote. However, only certain sera are capable of detecting this difference in expression. Typing sera have been prepared using the various donor-recipient combinations involving this locus in an effort to determine the factors influencing the ability of an isoantibody to detect gene dosage effects. The three genotypes which can be tested with each of the three specific sera have been compared, and the relation of the results to the question of dominance in a multiple allelic series involving blood groups has been examined.—The interaction of two of the alleles at this locus (Hg^A and Hg^D) gives rise to another antigen (I). Thus the heterozygote Hg^A/Hg^D has antigens A, D, and I. Quantitative hemagglutination tests indicate that the presence of the additional antigen decreases the amount of antigens A and D in the red blood cell. (This investigation was supported by a grant from the National Institute of Neurological Diseases and Blindness, Public Health Service.)

COHN, NORMAN S., J.W. Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *An analysis of the rejoining of X-ray induced broken ends of chromosomes.*—A series of X-ray fractionation experiments on seedling roots of *Allium cepa* has indicated that two distinct classes of broken ends of chromosomes, as judged by their average restitution time, are induced by irradiation. The experimental results on which this conclusion is based may be summarized briefly as follows. The frequency of chromosome exchanges at metaphase was determined for each of the following irradiations: a full continuous dose of 1600r, given in one minute; a single half dose of 800r, given in ½ minute; two fractions of 800r each with intervals between fractions of 2, 5, 10, 15, and 30 minutes, 1, 2, 4, 5, 6, 10, and 15 hours. The data obtained from these experiments indicate that within about 15 minutes a substantial fraction of the broken ends have rejoined, while some ends remained open for as long as 4 hours. At 6 hours, the frequency of exchanges was equal to that of twice the single half dose (or base line), and this frequency remained constant at all points beyond 6 hours. Preliminary experiments carried out prior to those just described were performed to determine: (1) the developmental stage at which irradiation would produce only chromosomal aberrations; (2) the time at which a relatively high frequency of aberrations are produced; (3) the length of time during which the aberration frequencies remain constant during root development. The procedures in the fractionation studies were contingent upon these results.—The demonstration of two distinct classes of broken ends with respect to their average restitution time suggests the possibility that the two types of broken ends may be qualitatively different. Experiments are currently underway to test the hypothesis that the more rapidly rejoining breaks are of ionic nature, whereas the more slowly rejoining types are of covalent nature.

CONGER, ALAN D., M.L. RANDOLPH and A. H. JOHNSTON, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Chromosomal aberration production by X-rays and by monochromatic 2.5-Mev and 14-Mev neutrons.*—Considerable attention has been devoted to comparisons of the relative biological efficiency (RBE) of fast neutrons to X-rays in causing biological damage. Differences in RBE are attributed to differences in the ion density or linear energy transfer (LET) (ions/micron, or kev lost/micron) of the radiation deposited in the tissue. Such neutron/X-ray comparisons have frequently overlooked the fact that the difference in ion density, and hence expected difference in RBE, may be as great within the fast neutron energy range as the difference between X-rays and energetic fast neutrons.—In a single experiment, Tradescantia inflorescences were separately exposed to three different radiations: to 250-kvp X-rays (hvl, 1.5 mm Cu), to monochromatic 2.5-Mev D, D fast neutrons, and to monochromatic 14-Mev D, T neutrons. The physical situations were well defined and tissue dose was carefully measured by several methods. Aberrations were observed in chromatid slides made 22–24 hr, and in chromosome slides made 4 days, after the irradiation. Comparisons were based on coefficients of aberration production per roentgen or rep derived from the six-point dose curves for each type of radiation.—Per unit dose, the more densely ionizing 14-Mev D, T neutrons (LET, 15 kev/μ) cause on the average about twice as much chromosomal damage as do the X-rays (LET, 2.6 kev/μ); the still more densely ionizing 2.5-Mev D, D neutrons (LET, 39 kev/μ), however, are twice as damaging as the D, T neutrons, or four times as damaging as the X-rays. The considerable change in chromosomal aberration RBE in the neutron energy range, a factor of two in these experiments, shows that neutron/X-ray RBE comparisons depend critically on the neutron energy or average energy, and that such comparisons are meaningless without knowledge of the neutron energy distribution.

DE SERRES, FREDERICK J., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *The origin of prototrophs in crosses of purple adenine mutants in Neurospora crassa.*—Linkage studies on a large number of phenotypically similar purple adenine mutants (derived from the same wild type strain) have shown that these mutants are located between the closely linked *hist-2* and *nic-2* loci on the right arm of linkage group I in a position corresponding to the *ad-3* locus. Heterocaryon tests, in all possible mutant combinations, have shown that these mutants comprise two physiologically distinct groups (A and B). Genetically marked mutant strains from each group were used for various intra- and intergroup crosses. In the intergroup crosses, adenine-independent progeny

occur at low frequency (1–10/2000), indicating close linkage of the two groups of mutants. Moreover, the origin of such isolates is associated with an exchange of the closely linked markers. These results have been interpreted as indicating that (1) the linear order of these two groups, from left to right, is *hist-2* A B *nic-2*, and (2) that the group A and group B purple adenine mutants have resulted from mutation at closely linked, but separate, loci in the *ad-3* region. Adenine-independent progeny have also been obtained from various intragroup crosses, but at lower frequencies (1–10/20,000). In preliminary crosses of the type *hist* A *nic* X+ A′ +, or *hist* B *nic* X + B′ +, the genotypes of the adenine-independent progeny were distributed among both the parental and crossover classes. The results of such crosses with mutants in either group indicate that the origin of protrophs is uncorrelated with recombination of adjacent markers if both parental strains are in the same group, whereas it is strictly correlated with an exchange of markers if both parental strains are in different groups.

DRAKE, BARBARA, Stanford University, Stanford, California: *Evidence for two loci governing para-aminobenzoic acid synthesis in Neurospora crassa.*—Eight independently derived para-aminobenzoic acid requiring strains were found to be associated with mutation at two loci in group V. The following results were obtained: 1633 (*pab-1*) × H-193 (*pab-2*) —20 prototrophs in 260 random isolate; 1633 × 71301 (previously thought to be at a third locus, *pab-3*)—9 prototrophs in 112 randoms; H-193 × 71301—0 prototrophs in 11,450 viable plated spores. (H-193 and 71301 do not form a wild type heterocaryon, but both form wild type heterocaryons with 1633.) 1633 × WSC-4-24 and WSC-4-24 × WSC-4-17ϕ—1 prototroph each among 15,000 or more viable plated spores; 1633 × S4298, 1633 × S4312 and WSC-4-24 × WSC-5-186—0 prototrophs in 660 or more viable plated spores for each. H-193 (*pab-2* × S4298—56 prototrophs in 740 viable plated spores. We conclude that 71301 is allelic to H-193 at the *pab-2* locus and that the others are allelic to 1633. (S4298 and S4312 are new strains obtained after X-irradiation.) Ascospores were plated in sorbose-minimal medium and viability estimated by layering representative plates with PABA. Mixtures of wild type spores and H-193 × 71301 spores showed no inhibition of wild type recovery by crowded mutant spores. Exhaustive attempts to demonstrate cross-feeding, both between the mutants and to aromaticless strains of Neurospora and *Aerobacter aerogenes* have been unsuccessful. (Work supported by the Life Insurance Medical Research Fund and the National Institutes of Health.)

EDINGTON, CHARLES W., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *A nonlinear frequency-dose relation for recessive lethals induced by X-rays in Drosophila.*—It has been found that the frequency of recessive lethals induced by X-rays in *Drosophila melanogaster* increases more rapidly with increasing dose than is expected on the basis of linearity. When the frequencies of recessive lethals induced by several doses of X-rays in Oregon-R and in *In(1)EN* males are plotted against dose and each dose curve is fitted to a weighted linear regression, both frequency-dose relations depart significantly from linearity. If these results are fitted to a quadratic equation, $y = a + bX + cX^2$, it is found that the Oregon-R data fit the quadratic equation, $y = 0.1494 + 1.5936X + 0.3653X^2$, and the *In (1)EN* data fit the equation, $Y = 0.4518 + 1.2789X + 0.4470X^2$ where y is the percentage of recessive lethals, and X is the dose of X-rays in kiloroentgens. This nonlinear increase in the frequency of recessive lethals induced by X-rays may be due to the increasing frequency at higher doses of one, two, or all of the following two-hit genetic effects: (1) two independent semilethals, which together act as a recessive lethal, (2) gross deficiencies, and (3) "position-effect" lethals, which are dependent on gross chromosome aberrations for their expression.

EISENSTARK, A., and C. KIRCHNER, Kansas State College, Manhattan, Kansas: *Genetic effects of chelation on phage-bacterial systems and on bacteria alone.*—Several investigators have observed chromosomal disturbance and increased crossover frequencies upon treatment of specimens with chelating agents. The effect has been attributed to the reaction of these agents with divalent cations that may bridge particulate genetic units. Since certain phenomena support the view that bacterial and phage genetic material interact, a possible method of elucidating the mechanism responsible for such interactions would be to alter their frequency by disturbing the bridges between bacterial genetic units with chelating agents. The agent used in this study was ethylenediamine

tetraacetic acid (EDTA). In addition to phage-bacterial systems, the genetic effects of EDTA on bacteria alone were also tested. Treatment resulted in a 2-fold increase of transduction of the *Try B-2* marker in *Salmonella typhimurium*, a 3-fold increase in *T3h* phage mutants, and a 2-fold increase in *T6h* phage mutants. A 750-fold increase in prototrophs was observed when EDTA-treated *Escherichia coli* K12 auxotrophs W1324 ($F+$) and W1393 ($F-$) were mixed; the effect was observed only upon treatment of the $F-$ strain, not upon treatment of the $F+$ strain. No effects were observed on mutation of *E. coli* B to B/3, reactivation of u–v'd phages or bacteria, induction in lysogenic cells, or mutation of bacteria to drug resistant types. These preliminary results indicate that genetic units in bacteria may be bridged by chelatable elements, and that treatment of cells with chelating agents may result in increased frequency of recombination, transduction, and phage h mutations.

EMMERLING, M. H., *Unequal crossing over between elements of the R complex in Zea Mays*. University of Illinois, Urbana, Illinois.—The occurrence of unequal crossing over within the *R* complex shows that this locus is a complex of two closely linked genes which retain their synaptic homology. On the assumption that these genic elements (P) and (S) pair indiscriminately, two types of derivatives are expected from homozygous (P)(S) as the result of unequal crossing over: (1) deficiency-crossovers (P)(−) where (S) is lost and (−)(S) where (P) is lost; and (2) duplication-crossovers (P)(S)(S) where (S) is duplicated and (P)(P)(S) where (P) is duplicated. In the present experiments these two classes of deficiency-crossovers have been identified in cultures marked at loci to the left and right of the *R* locus. Several R^g plant-color mutants were tested in heterozygous compounds to determine whether mutation of R^r to R^g occurs by a recessive mutation or by a physical loss of element (P). In the case of the compound $G(p)(S)k/g(P)(S)K$ (*K* and *G* represent distal and proximal markers), the expected types of unequal crossovers (seed-color) would include the apparent mutants $g(P)(-)k$ and $G(p)(-)K$. In addition mutants of non-crossover origin would include types $G(p)(s)k$ and $g(P)(s)K$. In a population of 89,550 gametes, this compound yielded 15 unequal crossovers and 7 non-crossovers. Of the 15 crossovers identified, 10 were of type $(p)(-)K$ and 5 were of type $(P)(-)k$. The non-crossovers included $3(P)(s)K$ and $4(p)(s)k$ mutants. The $G(-)(S)k/g(P)(S)K$ compound, on the other hand, should yield only $(P)(-)k$ unequal crossovers with none of the alternative class, $(p)(-)K$, since unequal crossing over occurs only when there are two or more synaptically homologous elements present. Out of 102,020 gametes tested, the $G(-)(S)k/g(P)(S)K$ compound produced 42 mutants, of which 24 were unequal crossovers and 18 were non-crossovers. The 24 unequal crossovers were all of $(P)(-)k$ class, The non-crossovers included $13(P)(s)K$ and $5(-)(s)k$ mutants. These results show that mutation of R^r to R^g yield mutants of constitution (p)(S) and (−)(S) which in turn produce seed-color mutants of types (P)(−), (P)(s), (p)(−), (p)(s), and (−)(s). (This work was done in collaboration with the late DR. L. J. STADLER.)

EPLING, C., D. F. MITCHELL, and R. H. T. MATTONI, University of California, Los Angeles, Calif., and Pennsylvania State University, State College, Pa.: *Correlated qualitative and quantitative changes in structural heterozygosity in the third and X chromosomes of Drosophila pseudoobscura during cyclic and directional fluctuations in nature*.—Systematic observation of the frequencies of structural homozygotes and heterozygotes in the third and X chromosomes of wild males of *Drosophila pseudoobscura* have been made at three stations for four years. A parallel directional change which has involved both chromosomes has occurred during this period at all stations. Cyclic seasonal changes of the homozygotes and heterozygotes of both chromosomes have occurred regularly at two stations but not at the third.—Analysis of the data show that heterozygosity in both chromosomes decreased simultaneously during the period of directional change at all stations and that it increased during the cyclic changes at the stations where cycles occur. The decrease of the sex ratio arrangement of the X chromosome during the directional change was correlated with a decrease of one arrangement of the third. The relation of the two chromosomes during the cyclic changes differed at each station. Both chromosomes contributed to the increase of total heterozygosity at one station but the increase at the other was chiefly the result of an increase of the sex ratio arrangement. Heterozygosity in the third chromosome remained at the same level. The increase of the sex ratio arrangement was corre-

lated at both stations with a similar qualitative change in heterozygosity in the third chromosome. No changes in either chromosome were observed at the third station.—The data are interpreted as evidence of interchromosomal effects of inversions on crossing over in the whole genome and their influence on adaptive recombination in accord with environmental differences and changes.

FABERGÉ, A. C., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Forecasting the rate of induced marker loss for maize endosperm.*—A consideration of data where several linked endosperm markers are followed simultaneously demonstrates that the overwhelming majority of losses from either X-ray or ultraviolet treatment follows chromosome breakage, and that point effects, if any, can only make a very small contribution. As a preliminary approximation, it is possible to construct a simplified model of chromosome behavior, on quite classical lines, which accounts reasonably well for the rather complex data (FABERGÉ, 1956, Z.i.A.V., 87: 392–420). Chromosome breaks are produced at a frequency approximately proportional to pachytene length. While LONGLEY (1950, Rep. Nav. Med. Res. Sec., Appl. 10, 1–60) has demonstrated departures from proportionality, these are not large. It follows that the relative sensitivity of markers, at a given dose, ought to be approximately predictable from a knowledge of the position of the locus on the chromosome arm, and the length of that arm. Localization of markers on the maize pachytene map is seldom very precise, and data on induced marker loss involve rather large statistical uncertainties. However, by use of only the constants obtained from the short arm of chromosome 9, roughly correct predictions are obtained for the markers *A*, *Pr*, *Su*, and *R*. Thus the proposed model, while admittedly oversimplified, seems adequate for use with the rough data available. These considerations also lead one to expect a mixed 1-event-2-event dosage effect curve for X-rays, which seems to be realized in practice. (Experimental work at the University of Missouri, supported by a research grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.)

FARNSWORTH, M. W., University of Buffalo, Buffalo, N. Y.: *Genetic control of terminal oxidation in Drosophila melanogaster.*—Cytochrome oxidase activity has been determined in homogenates of M(2)1[2] homozygous lethal larvae and their sibs, both the Minute heterozygotes and the wild type. Preliminary studies indicate that the cytochrome oxidase activity per microgram of protein is twice as great in the lethals as compared to the controls. The relationship between this enhanced activity and the complete failure of these larvae to grow is being investigated further.

FORD, LEE, University of Alberta, Calgary, Alta.: *A cytogenetic study of relative age of onset of spermatogenesis in the dog and rabbit.*—(*1*) In the dog. Study of spermatogenesis and chromosome morphology with reference to breed differences. Preliminary work indicates great differences in time of appearance of male cells in different strains, and this needs to be studied to see if there is any correlation with the onset of sexual behavior. The possibility that chromosome anomalies have arisen in the different breeds will be investigated. (*2*) In the rabbit. Study of regional differences in time and rate of maturation of the male germ cells in relation to morphology, viability, fertility, fecundity, sex behavior, and as affected by genetic and environmental influences such as mutant genes, inbreeding, age, season, disease, nutrition, and other environmental influences such as may have a bearing upon the use of these races in experimental breeding or experimental work on various constitutional diseases.

FORSTHOEFEL, PAULINUS F., University of Detroit, Detroit, Mich.: *Penetrance and expressivity of the luxoid and luxate genes in heterozygous C57BL mice.*—In heterozygous condition the luxoid and luxate genes produce a similar effect: preaxial polydactyly of the hind limbs. However the genes differ in penetrance and in expressivity. The maximum likelihood estimate of penetrance of the luxoid gene based on intercross and backcross material (a total of 1380 mice) is 0.8726. The intercross and backcross data were statistically homogeneous. The maximum likelihood estimate of penetrance of the luxate gene based on backcross material (504 mice) is 0.6623. These two estimates differ significantly.—Study of the expression of polydactyly in the luxoid and luxate heterozygotes

revealed a number of statistically significant differences. The luxoid gene most often affects both feet while the luxate gene usually affects one foot. The luxoid gene tends to range to the higher degrees of expression (7 toes) while the luxate gene is usually restricted to the lower degrees of expression. The luxoid gene tends to affect the left leg more often and more extensively than the right. This is the reverse of reported cases of polydactyly in mice including luxate.—The greater penetrance and the greater expressivity of the luxoid gene when compared to the luxate gene are probably aspects of a common phenomenon, viz., greater excess proliferation of mesenchyme in the preaxial portion of the limb bud of heterozygous luxoid embryos than is the case in heterozygous luxate embryos. (Supported by a grant from the National Science Foundation.)

FOSTER, M., Yale University, New Haven, Conn.: *Inherited variations in the pigmentary metabolism of mouse skin.*—It has recently become feasible to assay objectively and quantitatively two phases of normal mammalian melanogenesis, tyrosinase activity and initially present or newly formed melanin, with both assays performed consecutively on the same skin subsample. Manometric oxygen consumption measurements for equal weight subsamples of individual lyophilized skins incubated in phosphate buffer, tyrosine or dopa are followed by photoelectric turbidimetric measurements of alkaline thioglycollate suspensions of these subsamples. Thioglycollate suspensions of unincubated tissue serve both as controls for melanin formed during incubation and as measures of relative amounts of naturally occurring melanin in diverse genotypes.—The effects of separate and combined gene substitutions at the black-brown (*B*, *b*), pink-eyed (*P*, *p*) and maltese dilution (*D*, *d*) loci have been studied in this manner in non-agoutis. Preliminary results suggest that: (1) Although both assay methods generally agree, there is no simple correlation between observed melanogenic activity and amount of naturally occurring melanin. (2) On all backgrounds so far tested substitutions of *bb*, *pp* and *dd* for *B*, *P* and *D*, respectively, result in diminished natural melanin content. Combined substitutions show additive effects. (3) However, intense black (*B*−) skin is much less active than intense brown (*bb*), with *pp* and/or *dd* blacks and browns showing generally intermediate activities. (Work supported in part by NSF Grants G-517 and G-2110, and by an Institutional Research Grant from the American Cancer Society.)

FOX, A. S., and JEAN B. BURNETT, Michigan State University, East Lansing, Mich.: *The kinetics of protyrosinase activation in Neurospora crassa, Strain 15300.*—Preliminary genetic analysis of the difference in tyrosinase activity and specificity between the mating types of strain 15300 (albino-2) has suggested the existence of an enzyme precursor ("protyrosinase") (FOX 1954, Nature 173: 350). This hypothesis is supported by the observation that the tyrosinase activity of homogenates prepared at 5°C increases with incubation, and by the failure to obtain evidence of a dissociable inhibitor. The kinetics of tyrosine oxidation by such extracts are identical with those of purified enzyme, but the kinetics of dopa oxidation are not. The highest reaction rate is not proportional to substrate concentration and is observed only after a lag period during incubation with substrate. Initial reaction rates exhibit conventional kinetics. In the absence of substrate, tyrosinase activity increases during incubation, the rate of increase decreasing with time. The kinetics of this phenomenon are first order and directly proportional to homogenate dilution, suggesting a monomolecular or pseudomonomolecular activation process. The enzyme resulting from incubation at 22°C exhibits the same Michaelis constant as that initially present. The initial rate of activation exhibits a temperature coefficient of approximately 2.7 (15° to 25°C), but the maximum tyrosinase activity achieved is the same at all temperatures. A broad optimum at pH 5.0 is exhibited by the activation process, but this is probably confounded by irreversible tyrosinase inactivation at the lower pH's. The following models of activation seem possible: $P \rightarrow T$ or $P \rightarrow T + A$ (P, protyrosinase; T, tyrosinase; A, inactive byproduct). (Supported by research grants from the National Institutes of Health (C-2440) and from the American Cancer Society.)

FRANZKE, C. J., and J. G. ROSS, South Dakota State College, Brookings, South Dakota: *Colchicine induction of new true-breeding mutants from previously induced true-breeding mutants in sorghum.*—A sorghum variety, derived by selection from crosses of grain sorghums of approximately

its same height, was subjected to colchicine treatment. A slightly taller, true-breeding line was obtained immediately. When this was re-treated two different dwarf mutants were obtained which also bred true immediately. Re-treatment of these two mutants gave new true-breeding types, some of which resembled the original untreated variety. This study is further evidence for the mutagenic action of colchicine and for its immediate induction of apparent homozygosity.

FUERST, ROBERT, and T. C. HSU, The University of Texas M.D. Anderson Hospital and Tumor Institute, Houston 25, Texas: *Effects of 4-aminopyrazolo (3,4-d) pyrimidine on growth of Neurospora mutants.*—Growth inhibition of Neurospora Em5256A by the potential cancer-chemotherapeutic agent 4-aminopyrazolo(3,4-d)pyrimidine (abbreviated 4-APP) and some of its derivatives, can be partially overcome by a number of chemical substances. Among these are adenine, thiamine, thiamine pyrophosphate, pyridoxine, pyridoxamine, pyridoxalphosphate, caffeine, 4,5-diaminouracil, 2,4,5,6-tetraaminopyrimidine, cytidylic acid, desoxyadenosine, desoxyadenylic acid, ribonucleic acid, desoxyribonucleic acid and a series of active 4-APP derivatives. This inhibition is temperature dependent, and occurs best at an incubation temperature of 25°C.—2,6-Di(diethylamino)purine is itself very toxic. However, in the presence of 4-APP it partially relieves the 4-APP inhibition but exerts its own effect. Conversely, 4-APP relieves the inhibition produced by aminopterin or amethopterin.—Among a series of mutants tested in the presence of 4-APP, the thiamineless mutant 18558A proved most interesting. This strain is known to produce its own thiazole but requires pyrimidine. It was found to be mildly resistant to 4-APP. In fact, it appeared to be able to utilize adenine rather well instead of pyrimidine. Implications of these findings are of importance to intermediary metabolism in the formation and action of cocarboxylase, and in linking purine, pyrimidine, and nucleic acid functions to enzymatic processes. Adaptation experiments with the wild type strain Em5256A to increasing 4-APP concentrations have been successful in establishing 4-APP resistant strains.

FUNG, SUI-TONG CHAN, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *A major sex locus in Drosophila melanogaster.*—The dominant third chromosome gene *Hr* (GOWEN 1942) in *Drosophila melanogaster* affects only diploid females, producing sterile individuals with mixtures of male and female sexual organs. The third chromosome recessive *tra* (STURTEVANT 1945) is also sex-limited in effect. It does not affect males but females homozygous are transformed into sterile males. We have mated males with the *Hr* gene to females heterozygous for *tra*. Among the offspring, a specific sex type (*Sb Hr/tra*) was obtained. Morphologically, they differ from our hermaphroditic types (*Sb Hr/+*) or the transformers (*tra/tra*). The body size of these flies resembles females. Their external genitalia are male-like but appear retracted and have no visible claspers. On the first tarsal segment of the fore-leg, the sex combs consist of a row of 8–9 teeth. The internal genitalia are majorly composed of male sexual organs. Dosage effects were tested by introducing the two genes into triploids. *Sb Hr/+/+* individuals resemble normal triploid females. They are sterile and the fore leg has sex combs with 5-6 teeth. *Sb Hr/tra/+* flies resemble diploid hermaphrodites (*SB Hr/+*) but the internal reproductive systems are predominantly males. Their sex combs also consist of 5–6 teeth. *Sb Hr/tra/tra* flies resemble *Sb Hr/tra*, both externally and internally, and the sex combs have 8–9 teeth. The reproductive systems of flies having three doses of *tra* are similar to the homozygous *tra/tra*. They are indistinguishable from normal males. They do mate with females, but are completely sterile. The effect produced by the addition of the *tra* gene to our *Hr* shows that the two genes are allelomorphic and male-determining. With their presence, the diploid females can be shifted or transformed to various degrees from hermaphroditic types to almost complete males.

GARBER, E. D., and JOSEPH GORSIC, University of Chicago, Chicago, Ill.: *Interspecific hybridization in Collinsia Nutt.*—The plant genus Collinsia includes more than 20 species mostly of the western United States. The basic chromosome number is 7; tetraploid species have not yet been found. Interspecific hybrids involving about one-third of the species in the genus have been analyzed cytologically. One species, *C. sparsiflora*, appears to have undergone extensive chromosomal reorganization relative to two other species, *C. heterophylla* and *C. concolor*. Interspecific hybrids involving *C. sparsiflora* and either *C. heterophylla* or *C. concolor* display two reciprocal translocations and two paracentric

inversions. Hybrids between *C. heterophylla* and *C. concolor* yield only bivalents. (Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago).

GAULDEN, MARY ESTHER, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *DNA synthesis and X-ray effects at different mitotic stages in grasshopper neuroblasts.*—A study has been initiated to determine at what stage or stages of mitosis synthesis of deoxyribonucleic acid (DNA) occurs in the grasshopper neuroblast, to determine the effects of irradiation on this synthesis, and to determine whether radiation-induced mitotic inhibition can be correlated with an inhibition of DNA synthesis.—Grasshopper embryos were observed in living culture preparations. When a neuroblast entered a given stage of mitosis, C^{14}-labelled thymidine was added to the culture medium. When the cell reached the end of the given mitotic stage, the embryo was fixed immediately and washed with water to remove unincorporated thymidine. It was subsequently sectioned, stained with Feulgen reagent, and covered with stripping film. The cell originally observed in living condition was then examined for presence of grains in film above it. (Since fractionation of embryos revealed that the C^{14} was in the DNA fraction, it is assumed that uptake of thymidine indicates synthesis of DNA.)—Uptake of thymidine in neuroblasts begins in the middle telophase and continues into very early prophase. Maximum uptake occurs during late telophase and interphase, rate of uptake being approximately the same in both stages. Thus the stages of mitosis most sensitive to X-rays, namely middle and late prophase, are not involved in synthesis of DNA. Data accumulated thus far indicate that interference with uptake of thymidine occurs only in cells that have received a dose of X-rays much higher than that sufficient for blocking mitosis.

GILES, NORMAN H., and C.W.H. PARTRIDGE, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut: *Genetic and biochemical studies of mutants and revertants in adenine-specific strains of Neurospora crassa.*—A series of adenine-specific strains of *Neurospora crassa* has been obtained from X-irradiated and control conidia of a wild type strain (74A) utilizing the filtration-concentration technique. On the basis of heterocaryon and crossing tests, these mutants comprise two distinct types—group E (10 mutants) and group F (14 mutants). (The only previously known adenine-specific mutant type in Neurospora [ad 4-mutant 44206] falls into group F.) Biochemical studies indicate that the two types are involved in two sequentially related terminal steps in adenine synthesis, group E mutants being blocked in the conversion of inosine monophosphate (IMP) to adenosine monophosphate succinate (AMPS), and group F mutants being blocked in the splitting of AMPS to adenosine monophosphate (AMP) and fumaric acid. Of particular interest is the evidence that activity of the AMPS splitting enzyme, adenylosuccinase, is absent in extracts of all F mutants (and also in mutant 44206), whereas highly active enzyme preparations are obtained from wild type and from E mutants.—Preliminary biochemical and genetic studies have been performed on a series of 15 revertants (adenine-independent strains) obtained by ultraviolet irradiation of conidia from one F mutant. On the basis of crosses to wild type, all 15 revertants test as reverse mutations rather than as suppressor mutations. Biochemical tests indicate that adenylosuccinase activity has been restored in the revertants, although the level of enzyme activity appears to be generally below that of wild type. The present case thus represents a situation where forward mutation from wild type results in the loss of detectable enzyme activity, and reverse mutation in the restoration of this activity. (This research has been supported in part by a grant from the Atomic Energy Commission.)

GORSIC, JOSEPH, and E. D. GARBER, University of Chicago, Chicago, Ill.: *Genetics of Collinsia heterophylla Buist.*—More than 50 monogenic differences have been found in *C. heterophylla* (2n:14). Several multiple allelic series have been established. Characters used in the taxonomic treatment of the genus have been observed to have a monogenic basis in this species. A number of closely linked genes has been detected. (Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.)

GRELL, RHODA F., California Institute of Technology, Pasadena, Calif.: *Influence of a Y chromosome on the Dubinin effect.*—The influence of an extra Y chromosome on the Dubinin effect has been

measured for two translocations betwen chromosomes 3 and 4 — *T(3;4) 86D* (break points at 86D and 101) and *T(3;4) 89E* (break points at 89E and 101). The sequence of crosses was: XXY females (Y derived from Canton-S wild type stock) homozygous for the *dl-49* inversion in X and for *ci* and ey^R in 4 were crossed to *T(3;4) 86D* homozygous male or *T(3;4) 89E* $/ey^D$ male. The F_1 regular daughters were graded for the Dubinin effect by a modification of the system devised by STERN *et al.* (1946). The presence or absence of a Y chromosome in the latter females was then determined by mating them individually to *B; Sb/Xa* males (the difference in rate of non-disjunction of X's is extremely marked because of the heterozygosity for the *dl-49* inversion). At 20°C *T(3;4) 86D / ci* ey^R has a mean *ci*-index of 1.49 for XX females and a mean *ci*-index of 1.19 for XXY females (i.e., less extreme *ci* phenotype in the latter class). At 20°C the second translocation has a mean *ci*-index of 2.26 for XX and 1.72 for XXY. For each translocation the difference between XX and XXY is highly significant. (Females homozygous for the *ci* mutant and tested by the same method showed a slight enhancement of the mutant effect if a Y is present.) Thus an extra Y chromosome partially suppresses the Dubinin effect of each of these translocations. These results increase the likelihood that the Dubinin effect is a variegated-type position effect.

HERSKOWITZ, I. H., and S. ABRAHAMSON, Indiana University, Bloomington, Ind.: *Dependence of X-ray-induced crossover-like exchanges in Drosophila oocytes and oogonia upon radiation intensity.*—Among the exchanges which may be induced between homologous chromosomes following mutagenic treatment are "crossovers" (involving exchanges at exactly homologous loci) and "pseudo-crossovers" (exchanges at nearby but non-homologous loci). The exchanges between homologous chromosomes induced in previous radiation studies were usually considered as being crossovers produced by single hits.—In these experiments, 4–6-day-old virgins, whose X chromosomes were heterozygously marked (y^2 *v f car/y* v^+ f^+ car^+. *Dp* scV^1 y^+) were X-rayed with 3000r delivered either intensely (in about 2 minutes) or protractedly (in about a 7-hour period) and then mated to males bearing appropriate recessive mutants. Exchanges involving the X chromosome region between the loci of carnation and the duplication (virtually at the centromere) were determined by inspecting the F_1. In each of the 6 groups of eggs scored, laid 1–2, 3, 4–5, 6–8, 9–11, and 12–14 days after treatment, exchange frequency was less in the control than the protracted series and less in the protracted than the intense. For example: for eggs of days 1–2 these respective values were 3.1 ± 0.2%, 4.6 ± 0.3%, and 6.3 ± 0.5%, contrary to the accepted idea of no effect on "crossover" frequency for the first 6 days. For days 12–14 they were 2.1 ± 0.2%, 5.9 ± 0.3%, and 8.1 ± 0.3%. Although among treated eggs of the first 4 days some exchanges were X-autosome half-translocations, the data suggest that most of the exchanges in excess of the control value were pseudo-crossovers, whereas those in later-laid eggs were crossovers. The distinct intensity effect found for both early and later-laid eggs proves the induced exchanges are dependent upon a multi-hit event. (This work has been supported by a grant to DR. H. J. MULLER and associates from the United States Atomic Energy Commission (Contract AT(11-1)-195).)

HERSKOWITZ, I. H., H. J. MULLER, and JOHN S. LAUGHLIN, Indiana University, Bloomington, Ind. and Memorial Center for Cancer, N.Y.C.: *The higher efficiency of ordinary X-rays than of 18 Mev electrons in inducing chromosome changes when applied to Drosophila spermatozoa.*—Frequency of partial or complete losses of either paternal sex chromosome and frequency of translocations linking chromosomes II and III were determined following irradiation of inseminated females, using our standard "multi-purpose" stocks and techniques (see MULLER, 1954, D.I.S. 28: 144). 1350 rads (1500r) X-rays ("X") at 125 KVP were applied and the following doses of 18 Mev electrons ("E"): 600, 1500, 3750 rads. Loss frequencies were: for simultaneous controls, 0.4% (6/1608), for our accumulated control data, 0.17% (38/21,929); X, 1.4% (83/6018); E600, 0.3% (11/3295); E1500, 1.0% (90/8880); E3750, 2.9% (170/5252). Translocation frequencies were: X, 4.9% (98/1986); E600, 1.16% (36/3114); E1500, 4.1% (107/2620); E3750, 16.8% (203/1210). With rise of E dose, losses rose slightly faster than linearly and translocations rose as 1.5 power of dose, in good agreement with previous X-ray results. Converting the results to expectations for same number of rads, the X-rays proved very significantly more efficient. They produced about 1.5 times as many losses and 1.4 times as many translocations per rad at 1500 rads as the electrons. If ionization frequency for same number of rads is similar with these two types of radiation, these results would support the view that, at

least when X-rays are used, ionizations derived from the same electron track cooperate to an appreciable extent in the production of chromosome breaks. (Work supported by grant from U.S. Atomic Energy Commission (Contract AT (11-1)-195).)

HERSKOWITZ, I. H., and A. SCHALET, Indiana University, Bloomington, Ind.: *Half-translocations induced by irradiation of oocytes as a basis of dominant lethals in D. melanogaster.*—Among fertilized eggs laid within 4 days after X-raying oocytes a concentrated dose of 2300r causes 38% to fail to form larvae. At least half this mortality (say 20%) results from multiple X-ray hits. The present work tests the hypothesis that some of these multiple hits produce eucentric half-translocations which cause pre-larval death due to aneuploidy. Such aneuploid eggs would produce viable, euploid zygotes if fertilized by sperm of complementary aneuploidy.—The half-translocation tested for was one producing an egg hyperploid for all or almost all of IIR, when this was captured by a broken centromere-bearing chromosome, whose aneuploidy was compensated for when fertilized by sperm hypoploid for IIR. The irradiated females were *dp* (on IIL) *cn bw* (on IIR), the males were from a T(Y-II)C stock carrying dp^+, the exceptional F_1 being phenotypically dp^+ *cn bw*. After irradiation there were 11 such exceptions among 14,424 F_1 and none in 3097 controls. Since it was determined no more than ⅓ of the sperm would have been hypoploid for IIR, increasing the observed mutation frequency (0.08%) 5-fold yields that actually produced (0.4%). It seems probable that euploid half-translocations involving either arm of chromosomes II and III are equally likely and that these could arise either by capping or capturing. Accordingly, the total frequency of euploid half-translocations involving aneuploidy of an arm of the two major autosomes is roughly estimated to be 3.2% (0.4% × 4 × 2). This would comprise about 16% of the 20% multi-hit induced egg mortality. (This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

HILLMAN, RALPH, Yale University, New Haven, Conn.: *Temperature sensitive periods for the production of abnormal heads by Notch-Deformed.*—It has been established that the differentiation of the eye-antennal imaginal disc complex is labile as late as the end of the second larval instar. The phenotype studied here (first described by MORGAN in 1919) is associated with a Notch deficiency and is influenced by a modifier or modifiers at other loci. The results of this combination are variable. The lowest expressivity is a misplacement or fusion of the ocelli combined with rearrangement of the ocellar bristles. Greater expressivity is shown by a reduction in eye size which may be accompanied by duplication of all or part of the antenna on the affected side. The penetrance of this genetic effect is dependent upon the temperature at which the embryonic and larval stages of the mutant genotype develop. If the entire development of the embryo, larva, and pupa is at eighteen degrees centigrade as opposed to twenty-five degrees centigrade, the penetrance is raised from approximately ten percent to ninety-five percent. The lower temperature has been found to act on the developing imaginal disc before the end of the second larval instar at about forty-eight hours after hatching from the egg. The effect during the embryonic stage has also been studied, and it has been found, conversely, that higher temperatures during this period result in a greater penetrance and expressivity of the mutant phenotype. There is evidence to indicate that both the high temperature embryonic effect and the low temperature larval effect act on the same developmental system. Closer approximation of these temperature sensitive periods are now being attempted.

HINTON, TAYLOR, University of California, Los Angeles Calif.: *Differences in nucleic acid requirements of various strains of Drosophila.*—Raising *Drosophila melanogaster* under aseptic conditions, 54 genetically different strains were raised on a chemically defined medium with ribonucleic acid as an experimental variable. The control strain (Oregon-R) gave 81.6% pupation in 15.8 days when no nucleic acid was in the medium and 94.8% pupation in 11.0 days with nucleic acid. When the other strains were compared with the control, they could be placed into the following categories: (I) with nucleic acid present, A. normal pupation in the normal length of time (11 cases), B. lowered pupation and 4 (av.) extra days required (4 cases); (II) with no nucleic acid present, A. normal pupation in the normal length of time (13 cases), B. lowered pupation in the normal length of time (7 cases), C. normal pupation but 6–7 extra days required (2 cases), D. no pupation (2 cases); (III)

with nucleic acid present in part of the experiment but omitted in another part, A. no growth without nucleic acid, but normal with nucleic acid (5 cases), B. no growth without nucleic acid or extra amino acids (3 cases), D. no growth without nucleic acid as well as no growth when it is present (4 cases). Representative cases were selected and studied in more detail both biochemically and genetically. In genetical terms the analyses led to varied conclusions ranging from a heterochromatin effect in one case, to single dominant in another (as examples); and in biochemical terms, from a block in adenine synthesis in one case and an adenosine block in another, to amino acid-nucleic acid relationships in one case and purine-pyrimidine relationships in another.

Hsu, T. C., and Robert Fuerst, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston 25, Texas: *Biology of Neurospora crassa.*—Time-lapse, phase cinematography offers opportunities for some special phases of investigations on the biology of microorganisms. This is illustrated by a preliminary study on *Neurospora crassa.* Conidia spores and ascospores of strain Em5256A were dusted into simple hanging drop chambers which contained minimal medium. Motion pictures were taken of these preparations with accelerations ranging from natural speed to 960×. Germination of the spores and growth of mycelia could be observed from this film, and their rates can be measured quantitatively by subsequent analysis of single frames. Remarkable activities of protoplasmic flow were observed from most mycelia during the earlier hours of mycelial maturation. Cytoplasm, together with various granules, particles, and vacuoles could be seen to pass the septa until the vacuole became too large and the septum was plugged. The direction of the flow could be either from the tip to the base, or vice versa. It also might go from the main mycelium to the branches, or from the branches to the main mycelium. Conidia spores of the adenineless strain T34A germinate with a rate similar to that of wild type in minimal medium, but the mycelia died before reaching maturity. Spores of wild type germinated in a medium containing 4-aminopyrazolo(3,4-d)pyrimidine; but the mycelial growth seemed to be slower than that of the controls and many failed to mature.

Hyde, Beal B., University of Oklahoma, Norman, Okla.: *The effect of versene on the structure of plant chromosomes.*—It has been suggested that calcium, or possibly some other divalent cation, forms essential links between units of chromatin or molecules of DNA. To determine the immediate effect of a cation deficiency on chromosome structure, non-excised root tips of onion and *Vicia faba* were treated with .01M solutions of versene. The tips were fixed in a 3:1 alcohol-propionic acid mixture. Squash preparations were stained with a saturated solution of Sudan Black B in 45% propionic acid. Preliminary tests have shown that this stain is not effective for chromosomes after treatments such as Feulgen hydrolysis or lengthy alcoholic fixation.—The first noticeable effect (within 3 hours) of the treatment is suppression of anaphase movement of chromosomes. Accompanying this change is a marked swelling of the metaphase and late prophase chromosomes. This swelling is not uncoiling. The chromosomes take on an even reticular appearance. The fact that swelling is not accompanied by any marked loss of stainability accords with previous findings that versene treatment breaks chromatin into smaller units, not into its component parts such as DNA, histone, acid protein, etc. The swelling process will continue until discrete metaphase chromosomes can no longer be distinguished. Resting nuclei lost all structure to become uniformly fine granular.—Since treatments of this length are usually lethal to root tip cells, shorter (30–45 min.) sub-lethal treatments are now being used. These treatments slow division for several days but do not appear permanently to affect chromosome structure.

Jaap, R. George, and B. L. Goodman, Ohio Agricultural Experiment Station, Columbus 10, Ohio: *Weight variation in glands associated with antibody production in the fowl.*—The bursa of Fabricius and the spleen have been demonstrated to be associated with antibody production in the domestic fowl. At ten days of age both glands are extremely variable. The coefficient of variation for the spleen and bursa is 31 and 42 percent, respectively, as contrasted to 12 percent for body weight. Regression of gland weight in mg of body weight in gm is between 1 and 1.5 for the spleen as compared with 3 to 4.5 for the bursa.—At ten days of age about 8 percent of the variance in spleen weight has been found to be heritable in an interbreeding line. In contrast about 74 percent of the variance in

bursa weight appears to be genetic; the major portion contributed by the dam (21 percent) and by sire—dam interaction (53 percent).—Repeatability of progeny from the same mating was high. The variance between progenies of different matings was 72, 76, and 78 percent of the total for spleen, bursa and body weights, respectively. Two chicks of one sex gave 95 percent of the accuracy obtainable from twenty chicks.—Evidence for distinct heterotic effects of bursa growth have been obtained by comparing cross-line with intra-line, as well as from the high intra-line sire-dam interaction among progeny of the same dam. Spleen weight exhibited a distinct sire-dam interaction when cross-line were compared with intra-line progeny. There was no evidence of specific combining ability in intra-line comparisons of spleen weights.

KALTER, H., The Children's Hospital Research Foundation, Cincinnati 29, Ohio: *Variability of the presphenoid in the perinatal mouse.*—The alizarin-stained, osseous skull of the perinatal mouse contains several metrically and morphologically variable elements. This is especially true of of the presphenoid and vomer. Several dimensions of the presphenoid were measured in newborn and six-day old mice of the A/Jax, DBA/1, and C57BL/6Jax strains, in F_1 hybrids of crosses between these strains, and in offspring of several backcrosses. In the newborn the presphenoid was found to be smallest in the A/Jax mice (11.6 units coronally × 7.6 units sagitally; 100 units = 7.5 mm); largest in DBAs (43.4 × 13.0); and intermediate in C57BLs (35.2 X 9.7). The average size of the hybrid and backcross newborn presphenoids was in all cases between those of the parent-type newborn presphenoids. The extreme difference found between the A/Jax presphenoid "width" on the one hand and that of the DBA and C57BL on the other is due to the absence at birth in the first strain of the osseous alae orbitales and the minuteness or absence of the osseous postoptic roots, and is thus an apparently qualitative difference; whereas the difference between the DBA and C57BL mice is one of degree of size attained at birth by the osseous alae orbitales and postoptic roots, and hence is a quantitative difference.—By six days of age the dimensions of the presphenoid were 36.5 × 17.9 in A/Jax mice, 49.5 × 21.2 in DBAs, and 47.7 × 19.4 in C57BLs. Six-day old F_1 hybrid presphenoids were between these sizes. Despite the smaller inter-strain size differences at six days than at birth, characteristic morphological features make for ready identification of the presphenoids from the different strains. (Supported in part by a Postdoctoral Fellowship from the National Cancer Institute, U. S. P. H. S.)

KAPLAN, W. D., T. TANAKA, and K. TANAKA, Medical Research Institute, City of Hope Medical Center, Duarte, California: *The sterility component of X-ray induced dominant lethals in D. melanogaster.*—The determination of dominant lethals in *D. melanogaster* is based upon egg hatchability measurements. Brooding techniques, the mating of treated males to a succession of virgin females, permit the determination of mutation rates in sperm that were successively younger at the time of irradiation. However, there is no direct way in *Drosophila melanogaster* to distinguish between eggs that have failed to hatch because they remained unfertilized and eggs that have failed to hatch because development of the embryo has broken down as a result of an induced genetic event. For this reason a cytological study paralleling the genetic determination of dominant lethality was carried out for three broods of eggs following the irradiation of a group of adult males with a 2000r dose of X-rays. The data thus obtained provide evidence for the fact that in both the control and irradiated series unfertilized eggs contribute significantly to the dominant lethality rate as determined by hatchability tests.

KIMBALL, E., Clinton Experimental Farm, Clinton, Conn.: *Primary plumage pattern melanin restriction in Gallus.*—Wild type pyle-zoned restriction of black melanin is phenotypically limited to specific pterylar and subpterylar areas; similar comment applies to columbian belt-zoned restriction; whereas, extended black nonrestriction coincides with self-color. It has been suggested that the columbian factor R' is the hypermorphic allele, and the nonrestrictor r of extended black the hypomorphic allele of wild type R.—R', R, and r represent restriction quanta in a decreasing order of potency. Evidence is adducible to show R has identical potency in all feather germs, variable phenotypic effect depending upon extrinsic factors. The latter include physiological factors, such as pigmentation gradients, maturation effects, and gonadal hormone influences; morphogenetic factors

and specific genetic modifiers, as well as metabolic influences are also involved.—It follows that *R* has a single effect, melanin-restriction, identical in quantum in all feather germs; but extrinsic factors determine variable phenotypic manifestation of *R*-effect relative to expression-threshold. Wherefore, wild type pattern is not a phenotypic complex induced by action of a single gene, but rather by a galaxy of modifiers operative upon nonvarying *R*-effect.—The same rationale applies to analysis of *R'*-effect and *r*-effect. Moreover, the pigment substrate upon which *R*, *R'*, and *r* act (whether b-black, b'-black, or B-black) likewise determines variable phenotypic expression, e.g., *R* induces nonblack wing-bays in adult males carrying b-black, but wing-bays are solid black if the substrate is B-black.—*R*, *R'*, and *r* in all instances are genes with single effects, and do not in themselves determine complex phenotypes.

KIMBALL, R. F., NENITA GAITHER, and STELLA M. WILSON, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Postirradiation modification of X-ray-induced lethal and deleterious mutations in Paramecium aurelia.*—X-ray-induced lethal and deleterious mutations in Paramecium can be decreased by postirradiation exposure to H_2O_2, low temperature, starvation, or streptomycin. The last has been most extensively used. Selection can be excluded because there is little, if any, death or other form of elimination until the mutations are brought to expression by autogamy several days after treatment. The mutational process can be modified for at least two hours after irradiation but cannot be modified after the paramecia have divided. It is unlikely that four such diverse agents would all act directly upon the mutation process. All inhibit growth and division, and the modification of mutation is probably a consequence, in some way, of this inhibition. However, the connection is not a simple one because low temperature and starvation are effective inhibitors of growth but cause only a small and variable modification of mutation. Moreover, streptomycin *increased* mutation in a few experiments in which, for unknown reasons, it inhibited growth more completely than usual. Possibly there is some sort of balance between increase and decrease of mutation, the outcome of which depends critically on the amount of inhibition of growth and the stages in the division cycle that are affected. Thus the X-ray-initiated mutation process has been shown to be modifiable for at least two hours after irradiation, but the way in which this modification is brought about is not clear.

LEE, WILLIAM R., University of Wisconsin, Madison, Wis.: *Radiation induced dominant lethal mutations in the honey bee.*—A queen bee ordinarily lays fertilized eggs in worker (female) comb cells and unfertilized eggs in larger drone (male) cells. In these experiments queens were inseminated with sperm from unrelated males treated with gamma radiation from Co^{60}. The viability of the eggs in worker cells decreased with increasing dose until at 10,000r virtually no eggs hatched, indicating that almost every sperm carried at least one dominant lethal. However, when the dosage was further increased to beyond 35,000r the eggs began to develop normally. This must be the result of sperm inactivation since the eggs, even though in worker cells, developed into males. Therefore the sperm inactivation dose is several fold higher than the 100% dominant lethal dose, as in Habrobracon.—Nearly all the lethals caused death in the egg stage.—There was no significant fractionation effect, the percentage of dominant lethals being the same when 2,000r was given in a continuous dose as when given in two fractions separated by 1 or 4 hours.—The proportion of lethals in sperm did not change after one year of storage in the spermatheca of the queen.—The curve relating dominant lethals to dose is in quantitative agreement with those of Drosophila and Habrobracon. It shows a highly significant departure from linearity (after correction for natural mortality and saturation), but approaches linearity at low doses. This is consistent with the hypothesis that dominant lethals are due primarily to single chromosome breaks at low doses and multiple break phenomena at higher doses.

LEVINTHAL, C., and C. A. THOMAS, JR., University of Michigan Ann Arbor, Mich.: *Evidence for the maintenance of the physical integrity of the chromosome of phage.*—Molecular autoradiographic techniques have been developed for measuring the radioactivity of single virus particles. Using this method it has been shown that a large piece of DNA of an infecting phage T_2 is transmitted intact

to a single progeny virus particle. Crosses have been performed in which bacteria are multiply infected with P^{32} labeled phage of one genotype and non-radioactive phage of another. Using a host range mutant the progeny of the cross shows phenotypic mixing; the phenotype and the genotype of the particles do not correspond. However when the progeny are allowed to grow through a second transfer at low multiplicity of infection, the phenotypic mixing is removed. After this second transfer the phage are separated according to their adsorption phenotype and it is found that those particles with the high P^{32} content per particle are primarily in the fraction with the same phenotype as the radioactive parent.—These experiments show that the large piece of DNA transmitted intact to a progeny phage is associated with the host range marker. Further experiments are underway to test this association with other markers. Assuming that this association is found here also, then one can conclude that the large piece of DNA is the phage chromosome. The fact that it is transmitted to the progeny intact rules out crossing over as the mechanism for the production of recombinants in phage and suggests a mechanism involving the joining together of partial replicas of the parental structures. Thus the recombinants would be formed *de novo* under the cooperative control of two parental chromosomes.

LEWIS, E. B., California Institute of Technology, Pasadena, California: *An unstable gene in Drosophila melanogaster*.—"White-spotted" (*sp-w*, SHOWELL 1944; locus 1–1.5+) is a recessive mutant; *sp-w* females and males have fine-grained eye-color mottling with facets ranging from light orange to brown. By several criteria *sp-w* is not a variegated-type position effect. When *sp-w* is opposite apricot (*apr* or w^a), white (*w*) or cherry (w^{ch}) the eye color is homogeneous, brownish red. Attached-X studies place *sp-w* to the right of *apr* and probably to the right of *w*. *A pr sp-w* is white-eyed and *apr sp-w* / + + is wild type. *Sp-w* lies to the right of w^{ch} (which lies to to right of *apr*), based on two wild type crossovers and five complementary, presumptive w^{ch} *sp-w*, crossovers among about 557,000 offspring of *y ac* w^{ch} *fa* / y^2*sp-w* free-X females (heterozygous for autosomal inversions). Among these offspring were five products simulating wild type: three ("A-types") carried y^2; and two ("B-types") carried *y* and *ac*. Phenotypically, these types act like partial reversions and the A-type differs slightly from the B-type. Partial reversions have also twice appeared in progeny of *sp-w* homozygotes. Presumptive w^{ch} *sp-w* types have pale yellow eyes and w^{ch} *sp-w* / + + is wild type.—Thus, *sp-w* shows a cis-trans position effect with *apr* and probably with w^{ch}. *Sp-w* lies proximal to w^+ and is either an insertion of extraneous material or a mutant allele of a third pseudoallelic locus. Partial reversions of *sp-w* complicate the pseudoallelic analysis. Perhaps a similar type of complication exists in "allele" studies in Neurospora and Aspergillus.

LEWIS, H. W., and F. O. SCHMITT, Massachusetts Institute of Technology, Cambridge, Mass.: *Macromolecular organization of Drosophila salivary gland chromosomes*.—Experiments have been performed to test a proposed macromolecular model of chromosome structure. The model suggests that the built-in specificity of structure of each "species" of macromolecule, the number of different "species" of macromolecules (both DNA and protein) and the chemical environment determines the three dimensional pattern of weakly bound macromolecules. The macromolecules as individuals are not responsible for the cytological and genetic linear differentiation of the chromosomes, but rather these phenomenon result from the pattern of organization and interaction of these macromolecules. The experiments consist of disorganizing the structure of the salivary gland chromosomes of *Drosophila robusta* by treating these chromosomes with solutions of varying ionic strength and different types of ions as well as with reagents known to break hydrogen bonds. Subsequent return of the ionic environment to "physiological levels" results in the reorganization of band structure of the dispersed chromosomal material. Elaboration of the proposed model and its genetic implications will be discussed.

LIEB, M., Brandeis University, Waltham, Massachusetts: *Non-prophage forms of intracellular temperate phage*.—Previous studies of *Escherichia coli* K12S have shown that potential prophage can be eliminated by growing the bacteria at 43° immediately after infecting with the temperate phage lambda. When infected bacteria are incubated at 37°, the temperature sensitivity or pre-

prophage state lasts about 1 hour. The term preprophage has also been used by BERTANI to characterize superinfecting phage carried by bacteria lysogenic for a related phage.—Cultures of K12s lysogenic for lambda were superinfected with a mutant differing in plaque morphology. It was necessary to grow the lysogenic cultures in the presence of the related phage for several hours before superinfecting phage could be detected in a large fraction of the bacteria. The superinfected bacteria were then grown at 37° and at 43°. Superinfecting phage was not eliminated at 43° as would be expected if it were carried in the pre-prophage state.—At both temperatures a 2–3 fold increase in the number of bacteria carrying superinfecting phage was observed although the average number of superinfecting phages/bacterium was calculated from the percent of bacteria not superinfected to be approximately 1. Replacement of the prophage by superinfecting phage could not account for the increase. It is suggested that during exposure to superinfection, some bacteria may be multiply superinfected while others are physiologically immune to infection.

LIVINGSTON, CURT, and GEORGE YERGANIAN, (Introduced by A. G. Steinberg). Children's Cancer Research Foundation, Inc., Boston, Massachusetts: *Aberrations involving chromosome I of the Chinese hamster, Cricetulus griseus.*—Tumor CH-38MC is exceptional for the analysis of chromosome aberrations because of the 40-fold increase in endoreduplication, particularly after treatment with triethylene melamine (TEM) which results in the doubling of all initial breaks. Breakage of chromosome I (which averages 19 microns in length after colchicine pretreatment) by Nitromin and TEM is evidenced mainly at the centromere, in segments 1–3 and 5 microns from the centromere on the short arm, and at 4 and 8 microns from the centromere on the long arm.—Freezing the tumor at −76°C resulted in breakage at the proximal loci on either side of the centromere. Followed by endoreduplication (?) and differential rejoining, 2 of the 3 newly formed chromosomes have persisted now for 5 transplant generations. Contrastingly, the same breaks resulting from chemical influences failed to survive beyond a few cell divisions. Preferential or localized breakage by alkylating agents in regions possessing heterochromatic activity is, however, not fully corroborated, since chromosomes II, III, and VIII have breaks occurring at comparable loci.—A preliminary survey indicates that simple breaks at the four main segments of chromosome I could give rise to the heterogenic morphology displayed by the remaining 10 chromosome types. Pericentric segments, particularly the one on the short arm of chromosome I, have been demonstrated to be highly responsive to environmental alterations.

MARTIN, ALBERT, JR., and P. F. KOST, Veterans Administration Neuropsychiatric Hospital, Pittsburgh 6, Pa.: *Geographic endemicism in relation to a K-minus strain of Escherichia coli.*—Biochemical and serological analyses of selected samples of *Escherichia coli* from the Pittsburgh, Pa. region suggest endemic geographic biochemical variants within a single serological strain. According to classical analyses *E. coli* does not ferment adonitol, inositol, dextrin, cellobiose, and alpha-methyl-d-glucoside. The strain of *E. coli* isolated in this study is serologically K-minus, and biochemically does not conform to classical analyses in that it ferments cellobiose, dextrin, and alpha-methyl-d-glucoside in twenty-four hours with the production of acid and gas. A difference of possible significance between the postulated endemic strain of *E. coli* here reported and the ten biochemical types of KAUFFMANN (1954) is the almost universal production of gas by the former, and the absence of gas in the latter with the exception of those cultured in media containing mannitol and glucose.—Antisera were produced for each *E. coli* sample selected and employed in cross agglutination tests. Each of the test sera produced agglutination with each test organism in slide, tube, and growth tests. These serologic studies seem to indicate that the samples tested are K-minus and have a common O antigen. Further investigation of the strains of *E. coli*, their endemic geography, serology, biochemistry, and the classical methods for their identification may produce a more accurate scheme for strain and variant identification, and may produce areas of correlation between established criteria, and experimental serological and biochemical isolation.

MASSIE, WINFIELD, and MAX LEVITAN, Virginia Polytechnic Institute, Blacksburg, Va. and Woman's Medical College, Philadelphia, Pa.: *Structural and genic influences in recombination be-*

tween inversions.—Two strains (dubbed A and B) of *Drosophila robusta* homozygous for arrangements *XL-1, XR-2, 2L-3, 2R,* and *3R-1* were crossed, reciprocally, to strain C, homozygous for *XL, XR, 2R-1,* and *3R* and carrying either *2L* or *2L-1* on the second chromosome. All female offspring were heterozygous for the same inversions in both arms of X and in 3-right. Some were also *2L 2R-1/2L-3 2R,* the others *2L-1 2R-1/2L-3 2R, 2L/2L-3* constituting heterozygosity for simple inversion, *2L-1/2L-3* for overlapping inversions. When these females were mated, individually, to Standard males, the salivary gland chromosomes of their larval offspring provided data concerning crossing over between the proximal breaks of *2L-3 and 2R-1.*—Recombinants totalled 18.01% in 1077 larvae examined from eighteen *2L/2L-3* females, 18.29% in 853 larvae from ten *2L-1/2L-3.*—Females having a strain A parent produced 22.1% recombinants in 403 larvae when *2L-1/2L-3* and 24.0% of 472 when *2L/2L-3*; corresponding B females had 14.0% in 450 and 13.6% in 605. Also, results in the A series were homogeneous statistically; with strain B there was significant heterogeneity between individual females, and recombinants were rarer in C×B series (9.2% in 554 larvae) than in B×C (18.8% in 501).—The experiment suggests that, under similar karyotypic conditions in other chromosomes, genic (strain) factors influence recombination between *2L-3* and *2R-1,* whereas the complexity of the inversion loops (which should affect pairing in the region) does not. The proximity of the centromere to the *2L-3* break may explain the absence of gross structural influence here.

McKay, J. W., Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland: *Albino seedlings in chestnut.*—Open pollinated seed from 3 Chinese chestnut trees (*Castanea mollissima* Bl.) have given albino seedlings in the proportion of 4 to less than 1 percent. Crosses between the trees have increased the frequency of albinos to as much as 24 percent, but the increase is not consistent in all the crosses. In crosses with other trees albino seedlings have either failed to show or occur in very low frequencies. Data thus far are insufficient to indicate the method of inheritance.

Mertens, T. R., and A. B. Burdick, Purdue University, Lafayette, Ind.: *The use of X-irradiation to produce mutations in a polygenic system.*—X-irradiation was used to produce mutations in the polygenic system controlling earliness of maturity in the tomato. This trait exhibits heterosis in Lycopersicon, when divergent types are crossed. Furthermore, dominant genes seem to be the cause of heterosis for earliness in the tomato (Burdick 1954). If dominant beneficial growth factors can be radiation-induced, they should be detectable (by the heterosis produced) when an irradiated genome containing them occurs in an individual also having a normal, unmutated genome.—In these studies, seeds of autodiploid or inbred lines were irradiated and the irradiated and unirradiated genomes were combined by backcrossing an irradiated to a control plant. The earliness traits recorded for progenies so produced included (1) days to first flower on first inflorescence, (2) days to first flower on second inflorescence, and (3) days to first ripe fruit.—Two backcross progeny lines were isolated which were especially early. The one was derived from a seed of inbred Red Cherry line 018, which had received 6,000r of X-irradiation. This line was about four days earlier than the control line (018). A second line, derived from a 48000r X-irradiated seed of line 226, an autodiploid *L. pimpinellifolium,* was significantly earlier than its control for each of the three earliness traits. These two early lines represent two (3%) of the 65 R_1 plants from which backcross progenies were extracted.

Meyer, Helen U., and H. J. Muller, Indiana University, Bloomington, Ind.: *The high susceptibility of ring-shaped Y-chromosomes of Drosophila to loss both spontaneously and on irradition of spermatozoa.*—To determine loss frequencies of ring and non-ring Y's, males having Y^{Lc} (Muller 1948, D.I.S. 22: 73) and a normal Y ("Y^+"), respectively, and provided with an X having Y^S attached, were crossed to females with Novitski's X having both Y^L and Y^S attached. Eye pigmentation of sons carrying brown variegated (*bw^{VA}*) and vermilion was used as index of loss of sex-chromosome heterochromatin. Males 50–108 hours old were X-rayed with 4000r. For 0–30 hours after irradiation ("brood 1") and 30–72 hours ("brood 2") they were bred with successive females, which

were used until sperm depletion. Unirradiated controls were bred simultaneously. Light-eyed exceptional F_1 males were wherever possible bred to verify inheritability of the effect. Males carrying Y^{Le} gave the following estimated frequencies of loss (assuming the same proportion of inheritable cases among all observed exceptions in a given series as among those bred).—Controls: brood 1, 1.13% (total males 3120), brood 2, 1.31% (2621); irradiated, brood 1, 5.41% (1028), brood 2, 5.53% (1895). The corresponding results for males carrying Y^+ were, for controls: brood 1, 0.37% (3585), brood 2, 0.63% (3352); irradiated: brood 1, 2.06% (1479), brood 2, 0.81% (2509). Both spontaneous and induced exceptions are significantly higher with rings. Lack of drop in brood 2 of rings probably resulted from lesser virility and consequent lesser discharge of sperm in brood 1. If higher loss rate of ring chromosomes results chiefly from twisted restitutions, twistings must be extensive even in Y's, despite minute length (except for blocks) of their chromonema. (Work supported by a grant from American Cancer Society, on recommendation of Committee on Growth of National Research Council.)

MILLER, JAMES R., and MEREDITH N. RUNNER, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *The use of ovarian transplants in the analysis of maternal influence on body weight in mice.*—Ovarian transplants have been used to analyze the maternal influence on the growth curves of two strains of mice. The data consist of weights taken at birth and at 28 and 60 days of age. The F_1 between MCARTHUR's small strain (SM) and GOODALE's large strain (LG) was constituted and ovaries from females of each of the parental strains were transplanted into females of this F_1. Coat color genes served as markers to identify the true parentage of the young. In order to have some measure of the effect of the transplantation operation itself a series of intra-strain transplants (SM in SM and LG in LG) were made. Apparently the large strain is not sufficiently inbred to permit isografts since no results were obtained from the LG in F_1 and the LG in LG operations. Mothers belonging to the small strain were capable of supporting F_1 offspring that were considerably heavier than their own genetically small offspring. The small offspring from heavier F_1 dams were larger than controls from both transplanted and non-transplanted ovaries. The influence of hybrid mothers on genetically small mice was probably greater than a simple difference between the curves for the SM reared from ovaries grafted to F_1 mothers and the SM because the SM in SM group showed lower weights at 28 and 60 days. This reduction probably indicates that the operation *per se* affected the dams and resulted in the reduced weight of the offspring.

MITRA, S., (Introduced by J. H. TAYLOR), Columbia University, New York: *Types of aberrations produced by irradiation of premeiotic and meiotic chromosomes of Lilium.*—Pollen mother cells of *Lilium longiflorum* were examined following X-radiation at various stages from premeiotic interphase to two hours before anaphase I. Reciprocal translocations, which were chosen as representative of chromosome-type aberrations, could be scored at late diplotene. The only chromatid-type aberration that could be consistently distinguished from chromosome-type aberrations was a chromatid connection between non-homologous centromeres with an accompanying fragment. Connections between chromatids without free frgaments were scored as half-chromatid bridges. Transition from chromosome to chromatid-type aberrations occurs during late premeiotic interphase and leptotene. Chromatid-type aberrations first appear from irradiation of late premeiotic interphase and continue to form until a few hours before anaphase I. Half-chromatid aberrations increase from irradiation at late pachytene and reach a peak six hours before anaphase I.

MOHLER, J. D., Oregon State College, Corvallis, Ore. *Genotype and phenocopy frequency in Drosophila melanogaster.*—Studies were made upon the genetic basis of developmental homeostasis as measured by the incidence of heat induced crossveinless phenocopies (cvp). A limited range of differences was obtained among three wild type stocks established from flies in nature. Two highly inbred wild type lines were higher and one was lower in cvp frequency than the wild samples. F_1 hybrids between inbred lines gave three responses in the percent of cvp; a, higher than either parent (the two higher lines); b, equal to the parent of higher incidence; c, intermediate between the parents. Six generations of selection for increased cvp frequency from the F_2 of a cross of type c yielded positive gains, but selection from the same source for lowered proportion of cvp was apparently without

effect. No gains were obtained selecting either direction from the inbred parental lines. The F_1 between two lines selected for high frequency from one source exhibited a reduced cvp incidence. Spontaneous crossveinlesslike (cvl) were observed in nearly all crosses and lines. Only from those among flies from nature and to a limited extent from a female in a line selected for lowered cvp incidence were increases in the percent of cvl obtained by selection. No increased cvl followed selection for increased proportion of cvp. The results indicate that the genetic differences between lines are largely non-additive; however they are in some important ways inconsistent with a hypothesis that the developmental buffering in this case depends upon generalized heterozygosity.

MORTON, N. E., W. H. STONE, and M. R. IRWIN, Department of Genetics, University of Wisconsin, Madison, Wisconsin: *Linkage and fitness of cattle blood factors.*—Blood typing data accumulated by workers in our laboratory during the past 15 years were used for these studies. Erythrocytes of 5000 cattle were tested for more than 30 antigenic factors distributed among ten blood group loci (AH, B, C, FV, J, L, M, SU, Z and H′). Forty-four of the 45 possible linkage tests were carried out on 42 sire families of unknown linkage phase, each with 20 or more tested calves. Probability ratio tests of double backcrosses were set up on IBM machines to detect or exclude looser linkage than would be obvious by direct examination of the data. The maximum likelihood estimate of the crossover value was greater than 30 percent in all but four tests, for which the amount of information was small. None of the tests showed significant evidence for linkage of any magnitude. As far as can be determined from these data, the 10 blood group systems are independently inherited and provide markers for 10 separate linkage groups. Other analyses of the data from these 5000 animals showed no significant difference in the frequency of the blood factors between sires and dams, and no apparent effect of the presence or absence of any blood factor on fertility, as measured by the number of calves tested. There was no significant difference between the theoretical and observed homozygosity of the blood factors of 187 Holsteins with an average inbreeding coefficient of 25 percent. There was a 10 ± 4 percent deficiency in the proportion of calves with the blood factor from "incompatible" matings of positive sires with negative dams, as compared with the reciprocal "compatible" crosses, but this deficiency was not found in a smaller group of segregating families in which the heterozygosity of the positive parent was known by progeny test. There was no apparent difference in total number of calves from reciprocal matings, but this may be due to compensation for abortions. Although misclassification of a few homozygous sires of large families as heterozygous may account for failure to verify incompatibility in segregating families, the significance of incompatibility in reciprocal matings requires confirmation.

NANNEY, DAVID L., University of Michigan, Ann Arbor, Mich.: *Inbreeding deterioration in Tetrahymena pyriformis.*—In the course of inbreeding strains of variety 1, *T. pyriformis*, three types of anomalies appear with increasing frequency: 1) death at conjugation 2) cytological irregularities at conjugation and 3) abnormalities in micronuclear behavior, having their onset 50–100 fissions after conjugation, and resulting in the formation of inviable amicronucleate cells. Each of these anomalies has a complex, but apparently independent, genetic basis involving many individually negligible factors (polygenes). After passing through the various inbreeding crises strains may be obtained which are almost completely normal. The implications of these observations and the value of such studies employing microorganisms will be discussed. (Work supported by grants from the National Science Foundation.)

OSTER, IRWIN I., Indiana University, Bloomington, Indiana. *The frequency of induced lethals in morphologically different chromosomes.*—Irradiation with 2000r of spermatogonia of stocks containing either a rod X chromosome, a ring X, or an X (NOVITSKI's) with Y^S and Y^L attached, yielded the following induced lethal frequencies, 0.8% (41/4867), 0.1% (5/2475), and 0.2% (9/2146). (To compute these frequencies the control frequencies (0/1893, 1/705, 3/1531) were subtracted.) These stocks had been selected previously for equivalent productive capacities in order to minimize the possibility of biasing the results by forming "synthetic" lethals in those stocks bearing preexisting detrimentals. Parallel tests of translocations indicated by their low frequency that the cells tested were spermatogonia at the time of irradiation. The F_1 lethal tests of different P_1 males were re-

corded separately to avoid counting clusters of lethals as representing lethals of separate origin. The lower lethal frequencies obtained from the ring and NOVITSKI's X as compared to the normal X may have been caused by elimination of those lethals which were lost because of their association with structural changes. That is, whereas breakage of a normal X chromosome during interphase may usually result in restitution, a Y-derived region of the X may be less likely to undergo restitution, and a broken ring, by opening out, may have its restitution hampered. Persistence of open breaks would lead to isochromosome formation during the next mitosis. However, this interpretation meets difficulties in view of our having obtained sensibly equal frequencies (218/3789, 91/1545, and 98/1399 for the rod, ring, and NOVITSKI's X, respectively) for these three chromosome types after irradiation of more mature stages. (This work was supported by a grant to DR. H. J. MULLER and associates from the Atomic Energy Commission, Contract ZT (11-1)-195.)

PIPKIN, SARAH B., Rockville, Md.: *Sex ratios in wild populations of Drosophila.*—Sex ratios of the following Drosophila species were determined for pessimum populations (5 flies or fewer per collection) and expanded populations (6 or more flies per collection) in cases where populations of these kinds occurred in 6 areas in the Lebanon: immigrans, subobscura, buzzatii, funebris, hydei, busckii, and melanogaster-simulans. In Oahu, Hawaii similar studies were made for immigrans, ananassae, and mercatorum. In Truk, Eastern Caroline Islands, sex ratios of such populations were studied for hypocausta, ananassae, anuda, and melanogaster. Collection figures are based on from 8 to 20 or more collections per month, the collection period being 6 months in Hawaii, a year in Truk, and from 1 to 3 years in the Lebanon.—Species in which the ratio of males to females does not differ statistically from 1:1 both in pessimum and expanded populations include the following: buzzatii, busckii, and funebris at several areas in the Lebanon and melanogaster at Truk. Females outnumber males by approximately 3 to 1 in both pessimum and expanded populations of subobscura and immigrans in several areas of the Lebanon. Females outnumber males to a lesser degree in expanded populations of hydei in several Lebanese areas and busckii in one Lebanese area; hypocausta in Truk, spinofemora in Hawaii. In expanded anuda and ananassae populations in Truk and one Lebanese buzzatii population the males considerably outnumber the females. Experimental populations in the laboratory of several of the species tested yield 1:1 sex ratios. Preliminary tests indicate that greater female longevity and, to a lesser degree, earlier hatching time of females, help account for cases in which females far outnumber males in wild populations.

PITTENGER, THAD H., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Complementary cytoplasmically inherited mutants in Neurospora.*—Two different cytoplasmically inherited mutants, *mi-1* (poky) and *mi-3* (MITCHELL and MITCHELL, 1952; MITCHELL *et al.*, 1953) have been reported in Neurospora. These mutant strains are characterized by subnormal growth rates and defective cytochrome systems. In view of the complementarity shown by nonallelic nuclear genes in heterokaryons, it was of considerable interest to determine whether different cytoplasmic mutants could interact to produce a normal phenotype. With poky and *mi-3*, however, no satisfactory evidence could be obtained from mixed cultures to suggest any interaction between the two mutant particles.—More recently in this laboratory a third cytoplasmically inherited mutant, designated *mi-4*, has been found. In addition to other similarities both *mi-4* and poky are characterized by slow growth rates and deficiencies in their cytochrome systems that by present test are indistinguishable. When slowly growing strains of poky and *mi-4* are combined in a heterokaryon the two defective cytoplasmic determinants coexist in the cytoplasm, a heterocytosome, and the resulting culture has a nearly normal growth rate. It can be shown experimentally that the increased growth rate is the result of the mixed cytoplasm and not of nuclear interaction. The cytochrome oxidase activity of the heterocytosome is increased above that of either parent, although it remains far below that of wild type. These heterocytosomes, or those involving a normal particle and either of the mutants, are not permanently stable in certain genetic backgrounds and gradually revert to the slowly growing phenotype.

QUEVEDO, WALTER C., JR.,* and HERMAN B. CHASE, Brown University, Providence, R. I.: *The coat color of the Light mouse.*—The B^{lt} gene, presumably an allele of the black-brown locus, brings

about a clumping of eumelanin and a basal dilution of pigmentation in the hairs of B^{lt} homozygotes (Lights) and heterozygotes (Darks). The effect is more pronounced in B^{lt} homozygotes. With advancing age the coat color of Lights and Darks becomes progressively lightened, the later hair generations containing less pigment. Fewer active melanocytes are found in the hair follicles of homozygous Light mice than in the follicles of blacks and browns. Since no amelanotic melanocytes are found following the cessation of melanogenesis, it is suggested that the melanocytes are either lost from the hair matrix or become indistinguishable from the surrounding epithelial cells. The hair follicles of B^{lt} heterozygotes differ from those of the homozygotes, having active melanocytes present for a longer period of melanin synthesis and deposition. B^{lt}, however, apparently has no influence on the synthesis or deposition of phaeomelanin, the yellow-B^{lt} mice being indistinguishable from non-B^{lt} yellows. In contrast to B^{lt} PP mice where a clumping and basal dilution of eumelanin are found in the hair shafts, the gene p, when present either in homozygous or heterozygous condition (pp, Pp), reduces the degree of clumping and almost eliminates basal dilution. Apparently, p influences the rate of melanin synthesis and thus all subsequent pigmentary events, resulting in a more uniform pigmentation of the hair. (*U.S.P.H.S. Predoctoral Fellow).

REBHUN, LIONEL I., University of Illinois, Chicago, Illinois: *Electron microscopy of spermatids of Otala lactea.*—During sperm formation, the nucleoprotein of the heads of the late spermatids of the snail *Otala lactea* appears to be composed of axially oriented lamellae. In longitudinal sections these appear as sets of parallel lines 50–75 A wide separated by spaces of the same width. The lamellae seem to be attached to the thickened inner part of the double nuclear membrane. In cross sections above the level of the proximal centriole (which indents the base of the head) the lamellae appear as sets of parallel lines of the same width and separation as above. However, they may bend upon themselves many times and show no preferred direction of orientation. In sections through the base of the head (and therefore through the proximal centriole), the cross sections of the lamellae appear radially oriented. They may originate from the part of the nuclear membrane nearest the centriole or the part adjacent to the outer cytoplasm. In many cases the lamellae in this lower region make hairpin turns, returning again to the part of the nuclear membrane from which they originated. The radial orientation is thereby maintained. In regions above the centriole the lamellae may bend at any angle and a group of them may bend alternately in different directions. This leads to a less organized pattern. It appears probable that these structures are lamellae 50–75 A thick, 0.5–2 microns long, and 0.1–1.0 microns wide, attached to the nuclear membrane, with the long axis of the bent lamellae parallel to the long axis of the sperm head.—The development of these structures can be traced from the early spermatid. They begin to appear only after the mitochondria have begun to fuse and form the tail envelope. The mature sperm head, however, appears homogenous, the lamellae apparently being only transitory.

RUDKIN, GEORGE T., SALLY L. CORLETTE, and JACK SCHULTZ, Institute for Cancer Research, and The Lankenau Hospital Research Institute, Philadelphia, Pa.: *The relations of the nucleic acid content in salivary gland chromosome bands.*—An approach to the direct measurement of the DNA content per genetic unit is obtainable by the measurement of the ultraviolet absorbing substances in the bands of the giant chromosomes of *Drosophila melanogaster*. As a basis for the more precise analysis of individual loci, determinations were made of the most discretely resolvable bands in three different chromosomes, in 2L, 2R, and X. By a scanning microphotometric technique, estimations of the ultraviolet absorption of the individual bands were obtained. The extinction measurements range from .017 to .270 extinction units per band. The total of 59 measured bands fall into nine groups, separated by significant increments of absorption. The means of these groups form an arithmetic progression with a common difference of approximately .022 extinction units. The "unit" translated into terms of DNA is equivalent to 2.5×10^{-5} $\mu\mu g$ in the ultimate haploid strand, an order of magnitude one tenth the DNA per phage particle. It should be emphasized that this value is subject to many uncertainties, which will be discussed in detail. The data are not, however, inconsistent with the idea of a fundamental unit increased by "unequal crossing over", whose evolutionary consequences are complexes of tandem duplicate genes. The wide range of individual values, coupled with the known variation of recombination indices in different regions of the chromosome, emphasize

the unreliability of conclusions using average data of DNA per nucleus combined with recombination values for specific regions, to reach generalizations regarding the number of nucleotides required for genetic specificities. (Aided by grant C1613 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and an institutional grant from the American Cancer Society.)

RUSSELL, ELIZABETH S., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Inbreeding and selection for longevity of a lethal.*—A series of inbred lines favoring livebirth and survival of extremely anemic *WW* individuals was developed from a heterogeneous stock by repeated brother-sister mating of *Ww* heterozygotes, combined with mild selection among matings and periodic severe selection among portions of the pedigree. Ten lines were carried to F_{10-12}, and the four best of these have been continued to the present (F_{20-26}). In the initial stock 18% of liveborn offspring were *WW*, which survived for an average of 4.5 days. At the most recent calculations the percentages of *WW*s among liveborn, and their average life-spans are as follows: B-strain: 20.5%, 7.2 days; C-strain: 20.7%, 11.6 days; H-strain: 19.3%, 5.0 days; and K-strain: 18.7%, 9.7 days. This contrasts strongly with behavior on a C57BL/6 genetic background: *WW* 4% of liveborn, average survival 2 days. Adult *WW*s have been obtained occasionally in the C-strain, less frequently in the K-strain, and once in the B-strain. Other characteristics of these *WW*-favoring lines will be discussed. These four inbred lines, together with their F_1 hybrid matings with C57BL/6-W^v, which produce healthy severely anemic WW^v offspring, are essential to progress in study of manner of action of *W*-series genes.

RUSSELL, LIANE BRAUCH, and MARY H. MAJOR, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *A high rate of somatic reversion in the mouse.*—The spontaneous color mutation pearl (*pe*) is stable in its strain of origin, C3H. However, in another background, strain 201 (which is homozygous pearl), somatic reversions to full-color are frequent. The incidence in the first 360 animals was 3.7% in males, 10.1% in females. Most frequently, the spots occupy about 0.1% of the coat and, in those cases, germinal tissue was never found to be moasic. Five animals, however in which the proportions of full-colored fur were about 5, 50, 80, 100, and 100%, produced, respectively, 18.2, 52.7, 43.6, 38.5, and 36.2% full-colored young. Since the two completely full-colored animals produced significantly less than 50% full-colored young, it is concluded that they, too, were due to somatic, rather than germinal, events. Apparently, these events can occur early or late in development.—The reversion can be made homozygous and is fully viable. Presumed heterozygotes yield 1:1 ratios when crossed to unrelated *pe/pe*.—It was shown by appropriate crosses that the reversions are due to events at the *pe*-locus itself, or within 0.4% crossover units from it. The hypothesis is being tested that reversions are due to somatic crossing over between pseudoalleles. This explanation, however, seems rather unlikely since strain 201 is already somewhat inbred and the *pe*-region presumably homozygous in some sublines. It is, therefore, tentatively concluded that reversions are due to somatic reverse mutations at the pearl-locus.

RUSSELL, W. L., Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Lack of linearity between mutation rate and dose for X-ray-induced mutations in mice.*—Wild type male mice were exposed to a single dose of X-rays and, after their period of temporary sterility, mated to a stock homozygous for seven autosomal recessive visible genes. Control, unexposed, males were also mated to the test stock. Offspring were observed for mutations at the seven specific loci. The experimentally induced mutations recovered in this way come from cells irradiated in spermatogonial stage. The mutation rates obtained from the control males and males exposed to 600r and 1000r depart significantly from a linear relationship with dose. The point estimate of the mutation rate at 1000r is only about one third of that expected from a linear extrapolation of the 0r and 600r rates. One possible way of accounting for the departure from linearity is to assume that there is heterogeneity among spermatogonia with regard to radiation sensitivity, and that sensitivity to damage that results in cell death is positively correlated with sensitivity to mutation. Thus, at the higher dose, more of the sensitive spermatogonia would be killed and the mutation rate in the more resistant cells that survive would be lower. The amount of cell death observed in mouse spermatogonia exposed to doses in the 600r to

1000r range is consistent with the above hypothesis.—A 300r experiment, started after the above lack of linearity was found, has, so far yielded a mutation rate that does not depart significantly from a linear interpolation between the 0r and 600r points.

SAMPSON, D. R., Horticulture Division, Central Experimental Farm, Ottawa, Canada: *Genetics of self-incompatibility in broccoli.*—The self-incompatibility mechanism of broccoli (*Brassica oleracea* var. *italica*) is under the control of a single major gene with multiple oppositional alleles. The pollen reaction is sporophytically determined. Three sorts of gene interaction, independence, dominance, and incomplete dominance, were found in heterozygotes of the four *S*-alleles analyzed.

SAND, SEAWARD A., The Connecticut Agricultural Experiment Station, New Haven, Conn.: *Correlated effects of temperature on somatic and germinal mutation in a clone of variegated Nicotiana.*—A Nicotiana clone, S5230-5, selected from cultures of interspecific hybrid origin was employed. Flowers of this clone are variegated, showing sectors of speckled tissue on the normally red petals. Self-pollination yields progeny of which about 25% bear speckled flowers, indicating with other tests that the parental clone is heterozygous (v_g/v_a) for the speckled gene. Variation in the frequency of speckled somatic sectors and of speckled progeny is assumed to reflect alteration in the frequency respectively of somatically expressed and germinally transmitted products of mutational events at the *v* locus.—Clonal replicates were cultured at 50°, 60°, 70°, and 80° F constant temperatures. Samples of flowers from each temperature treatment were scored for their frequency of somatic sectors, and other samples of flowers were self-pollinated to produce seed at each temperature. The resulting progeny families were scored for their frequency of speckled individuals.—The data indicate that the parental somatic sectoring frequency increases with temperature. An average increment of 18% of the mean frequency was obtained for the temperature range employed. The progeny segregations from seed set at the different temperatures also differ, giving higher frequencies of speckled offspring from the higher temperature treatments. An orderly increase from 15% to 37% speckled progeny was detected for the range of temperature treatments tested.—Thus the speckled locus or a related physiological system, previously shown to be hypersensitive to gamma irradiation level at low dosage, is also mutationally responsive to differences in environmental temperature.

SCHURIN, MIRIAM F., and HEWSON SWIFT, University of Chicago, Chicago, Illinois: *Fine structure of Drosophila salivary gland nuclei.*—Thin sections of *D. virilis* salivary glands have been studied with the electron microscope. Grids have been treated with heavy metals to increase electron density of certain nuclear components. Light microscope comparisons have been made on thick sections stained for nucleic acids and proteins. Chromosomes were seen to contain numerous longitudinal fibrils 10 to 20 mμ in diameter, in both band and interband regions. Fibril structure in a few regions was suggestive of a tightly coiled helix. In some areas electron dense material formed lateral projections from the fibrils at intervals of about 15 mμ. Density was increased in the band regions by uranyl staining. A few bands were made up of electron dense spherical bodies with diameters up to 300 mμ. Vesiculate and lamellate structures were also seen, in contact with the chromosomes, the latter resembling the cytoplasmic lamellae of the ergastoplasm. In young (4 day) larvae filamentous interchromosomal material was radially arranged around nucleoli and around some chromosomal bands. These filaments could occasionally be traced through the nuclear membrane into the cytoplasm. The nuclear membrane was largely made up of densely packed contiguous annuli, each about 100 mμ in diameter. Where chromosomes were against the membrane, chromosomal material occasionally seemed continuous with electron dense projections of the annulus wall. Nuclear membrane, interchromosomal filaments, and a few chromosome bands, gave positive cytochemical tests for RNA. The localization of DNA, histones, and non-histone proteins, has been considered in relation to chromosome fine structure. (Aided by grants from the U. S. Public Health Service (C-1612), and the Abbott Memorial Fund.)

SHIFRISS, O., The Weizmann Institute of Science, Rehovot, Israel: *Precocious differentiation in Cucurbita pepo.*—As predicted (J. Heredity 46: 219), a unique monocolored yellow-fruited race was developed, known as Precocious Yellow. Whereas in Precocious Yellow the differentiation of the

yellow color occurs extremely early in ontogeny, long before anthesis, and is conditioned by gene *B*, in standard varieties it occurs at or sometime after anthesis and is conditioned by gene *Y*.—The so-called bicolor variation is brought about either through heterozygosity of *B* or, in *BB* individuals, through certain gene substitutions in the rest of the pigment-controlling genotype. These changes, and perhaps others, provide the conditions which delay the differentiation of the yellow color during the preanthesis period; the later the differentiation occurs, the smaller the yellow area on the upper portion of the fruit, the lower portion remaining green. The observations suggest that precocious differentiation is subject to a gradient existing between the proximal region and the distal end of the ovary and that this gradient gradually disappears before anthesis.—Gene *B* is stable in regard to fruit pigmentation, at least in dominant homozygotes, but is unstable in respect to its action in leaves; producing intermittent yellow spots on leaves which appear at early stages of plant growth. In unselected bicolor-fruited varieties the locus involved is unstable in both fruits and leaves.

SHOWALTER, A. M., Madison College, Harrisonburg, Va.: *The inheritance of fruit shapes in Cucurbita moschata, var. Golden Cushaw.*—Ten fairly distinct true breeding subvarieties have been obtained from a single ancestor by inbreeding and selection through fifteen generations. These subvarieties differ widely in shapes of fruits but are essentially alike in vine and foliage. F_1 hybrids of these subvarieties generally show some degree of hybrid vigor and bear fruits of shapes intermediate between the shapes of the parental subvarieties. F_2 populations generally are more variable than F_1 and are difficult to score since both size and shape of fruits are affected by environmental conditions. It seems reasonable to postulate a large number of pairs of alleles concerned with fruit shape, too many for simple Mendelian analysis.—One cross is of particular interest. The F_1 hybrid plants of this cross bear fruits with necks conspicuously longer than the neck of either parent. Backcrosses of these F_1 hybrids by the parental subvarieties give progenies showing approximately equal numbers of plants similar to the F_1 and to the backcross parent. This is interpreted provisionally as a case of hybrid vigor attributable to a single pair of alleles. It is hoped that larger populations of test progenies can be grown and scored in 1956.

SILVERS, WILLYS K., Brown University, Providence, R. I.: *The differentiation of melanoblasts from different genotypes of the mouse when transplanted to the spleen of adult mice or to the coelom of the chick embryo.*—To determine the site of action of various genes concerned with pigmentation in the mouse, somite regions were isolated from 8 to 13 day old embryos. These were transplanted to the spleen of adult hybrids, to the spleen of isogenic animals differing in at least one color factor, or to the coelom of the chick. The following genotypes were investigated: *aaBB*, *aabb*, A^vaBB, A^vabb, *AABB*, *aabbdd* and $aaB^{lt}B^{lt}$. Melanocytes containing black granules were observed within and outside the graft area when the transplants originated from animals of *aaBB* and *AABB* genotypes. Brown pigment granules occurred in melanocytes derived from *aabb* and *aabbdd* neural crest. Pigment granules, different from black or brown, were found in light ($aaB^{lt}B^{lt}$) melanocytes occurring within and outside the graft area. Melanoblasts from A^vaBB and A^vabb grafts differentiated into black and brown melanocytes, respectively, whenever they occurred outside of hair bulbs. Typical yellow pigment was observed within the hair shaft and bulbs of these grafts. Neural crest from *aabbdd* embryos gave rise to morphologically distinct melanocytes, characterized by a clumping of pigment granules, within and outside the graft area. In every case the quality of pigment observed outside the transplanted region was identical with that in the ear or tail skin of the genotype investigated. The results indicate that the *B* and *D* loci act autonomously within the pigment cell, whereas agouti and yellow pigmentation must be attributed to a specific action of the *A* locus on the environment of the melanocyte in the hair bulb. (Supported by a Postdoctoral Fellowship from the National Cancer Institute, United States Public Health Service.)

SMITH, LOIS JEAN, FAY A. LAWSON, and E. S. RUSSELL, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *The implantation of normal blood forming tissue in anemic hosts with persisting modification of host phenotype.*—F_1 segregants (WW^v, anemic; Ww, W^vw, and ww, normal) from crosses between mice of the $C_{57}Bl/6$—W^vw strain and Ww mice from two different inbred lines

were subjected to X-radiation of 200r. This was followed immediately by intravenous injection of 4×10^6 or 8×10^6 cells from $C_{57}Bl/6$ 15½ day old embryo livers. By 18 days, the hematocrit levels of the anemics with either type of genetic background were raised to the preradiation level of their normal sibs. Determination of mean cell volume indicated implantation of the *ww* hematopoietic cells. (WW^v anemia is macrocytic and normochromatic.) Anemic controls (irradiated and not injected) from the same two crosses reacted differently to this level of irradiation. In one cross, the majority was dead by the 18th day, while in the other, the majority has survived to 60 days. The survivors returned to their preradiation hematocrit levels or slightly lower ones. Sibs of the anemics returned to their characteristic hematocrit levels; those injected, within 18 days; those not injected, more slowly. Injection of isologous 15½ day old lethal anemic *WW* embryo liver cells into non-anemic *ww* adults of one of the *Ww* strains following X-radiation of 400r, provided no protection. These data indicate that the anemia of WW^v animals is determined by the genotype of their hematopoietic cells and is not imposed by the physiology of the mouse as a whole.

SMITH, STANLEY G., Forest Insect Laboratory, Sault Ste Marie, Ontario: *Intraspecific changes in chromosome number and morphology.*—Three males of a lady beetle, *Chilocorus stigma* (Say), collected near Sault Ste. Marie in 1955, were heterozygous for one, two, and three centric fusions, respectively (2n = 24–26), and owing to a segmental deficiency, one autosomal bivalent in the third male was heteromorphic. Ignoring the sex chromosomes (neo XY) and a supernumerary chromosome in each, the species in Ontario thus comprises potentially 81 chromosomally distinct types. In California, sampling between Marysville and Santa Barbara has revealed only double-fusion homozygotes (2n = 22), heteromorphic autosomal bivalents and supernumeraries being absent. In central Florida, only single-fusion heterozygotes occur in the two populations sampled (total, 38♂♂), but supernumeraries, of two morphological types, increase the chromosome number of most individuals from 25 to as high as 28. These observations, assessed in the light of the cytology of the tribe and of the family in general, establish a new principle in evolutionary changes in chromosome form and number, and provide a mechanical basis for the origin of supernumerary chromosomes.

SONNEBORN, T. M., Indiana University, Bloomington, Ind.: *Paternal age and stillbirth rate in Man.*—Induction of chromosomal aberrations by aged cytoplasm in Paramecium led me to investigate the possibility of a comparable effect in Man. With John Custer, a pilot study was made of the relation of paternal age to stillbirth rate, using data obtained in LaPorte, Indiana. The strong paternal age effect indicated by that study was confirmed on a large scale by calculations on data in the U. S. Birth, Stillbirth and Infant Mortality Statistics for 1935 and 1936: for mothers of any 5 year age class, the stillbirth rate rises significantly with age of father, except that the youngest fathers also had a high rate. The latter might be due to the higher rate at the first pregnancy. Parity, however, can hardly account for the consistently higher percentage of stillborn children when the fathers are over 44 than when fathers are 25 to 34, regardless of maternal age. For mothers in successive 5 year age classes, beginning with 15–19, the percentages are, respectively, 4.21 vs. 3.34; 3.96 vs. 2.74; 3.84 vs. 2.90; 4.24 vs. 3.37; 5.42 vs. 4.69; and 6.15 vs. 5.61. (Data for male children; data for females show similar differences with lower absolute values.) At this point, I found that YERUSHALMY (1939) had noted a paternal age influence in the U. S. data for 1931–1935 and that PENROSE (1955) interpreted YERUSHALMY's data to indicate increasing lethal mutations with age. Attempts are in progress to supply the following needed information: the parity effect; comparison of early (chromosomal aberrations?) with late (point mutations?) terminations of pregnancy; and comparison of stillbirths presumably due to zygotic constitution with those presumably due to maternal or extrinsic causes. (Supported by the Rockefeller Foundation, the Atomic Energy Commission, and the American Cancer Society.)

SPIESS, ELIOT B., University of Pittsburgh, Pittsburgh 13, Pennsylvania: *Relative adaptive values of chromosomal arrangements from experimental populations of Drosophila persimilis.*—The Whitney, Klamath, and Mendocino arrangements of chromosome III of *D. persimilis* taken from a locality at 8000 ft. in Yosemite Nat'l Park were tested in all combinations by initiating laboratory populations

at 15°C with alternate high and low frequencies of the competing arrangements. In two cases (WT vs. KL and WT vs. MD) WT is superior to the competing arrangement but eventual equilibrium is established, indicating superiority of the heterokaryotype; in each case however the relative adaptive values are not constant over the entire course of frequencies. Duplicate cages with identical strains but with different initial frequencies produce nearly identical results. KL at frequencies lower than 45% approach 30% equilibrium; while above 45% KL, populations approach a plateau, or temporary equilibrium, at that frequency. If MD is high relative to WT initially, the curve of loss in frequency of MD indicates a tendency towards elimination, but at 25–30% MD increases and equilibrium becomes established. Apparently relative adaptive values of karyotypes are not constant over the entire range of gene frequencies but are dependent upon the relative frequencies of those karyotypes in the population. KL and MD chromosomes when competing form adaptively equal karyotypes: cages with alternative frequencies consistently show no significant changes in relative frequencies. These arrangements rarely occur except in heterokaryotype with WT in the locality from which these strains were obtained and probably are not coadapted. Populations containing all three arrangements come to equilibrium more slowly, especially those with both high KL and high MD. It is difficult to interpret these results without assuming that relative adaptive values are determined to some extent by relative frequencies of karyotypes in the same population. (Work supported by a grant from the U. S. Atomic Energy Commission, Contract AT(30-1)-1775.)

STADLER, JANICE and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Significance of all body cells to genetic resistance to disease.*—Mice of 5 strains were exposed to 4 doses of partial to whole body irradiation, a total of 25 treatments. The mouse strains were genetically differentiated for typhoid resistance in the order S, Z, K, Q and Ba. X-rays were from a Maxitron operated at 250 pkv, 30 ma, filtered by 0.25 Cu and 1 Al, the doses being 0, 320, 480 and 640r. The regions of exposure were divided into thirds of the body—head, mid, rear, head mid, head rear, mid rear and whole body. There were at least 50 mice balanced for sex in each of the 125 experimental groups. Shielding was done with ⅛ inch lead. Mice surviving 15 days post irradiation were inoculated with virulent *Salmonella typhimurium*, 11C in doses partially adjusted for the known susceptibilities of the strains; S, Z, K 200,000; Q 2,000 and Ba 200 organisms. Deaths were recorded daily for 21 days. Sexes responded equally to the radiation and disease. Large strain and dosage differences were observed. Shielding the different thirds of the body protected the mice in varying degrees. The depression of resistance was greatest when the mid region was irradiated and least when the rear region was exposed. The effects of the irradiation on resistance was nicely linear and additive until whole body irradiations were reached. When full account was taken of the effects of the irradiations to the head, mid and rear there still remained a lowering of resistance equal to more than twice that of the combined parts. This further decrease in resistance supports the view that when there were no body cells undamaged by the irradiation there were no cells which could function normally in the resistant process. This suggests that all cells of whatever type found in these regions were active in recovery of the disease resistance processes.

STALKER, HARRISON D., Washington University, St. Louis, Mo.: *Selection within a unisexual strain of Drosophila.*—A unisexual, automictic strain of the normally bisexual species, *D. parthenogenetica* has been maintained for 80 generations. During this time the rate of normal development of unfertilized eggs has risen from 0.08% to 1.55%. This change occurred within the first 17 unisexual generations, thereafter there has been no increase in egg hatch, suggesting effective strain homozygosity for this character. The vigor of the adults, as measured by percentage survival through the first 20 days of adult life has declined from 96.5% in the first unisexual generation to 27.8% in the 80th generation. It is suggested that this decline in vigor is partly due to the stringent selection for egg hatching, with a relative relaxation of selection for viability and longevity. Crossing between generation 55 unisexual strain females and males from an unselected bisexual strain, followed by several generations of bisexual reproduction and then extraction and reselection of a new unisexual strain resulted in further increase in rate of egg hatch from 1.55% to 3.06%, and a rise in adult survival rate from 27.8% to 84.6%. These data emphasize the importance of residual bisexuality as a source of genetic recombination during the evolutionary development of parthenogenesis.

STEFFENSON, DALE, LULU F. ANDERSON, and STUART KASE, Biology Department, Brookhaven National Laboratory, Upton, N. Y.: *Crossing over studies with Drosophila and maize.*—Recent studies have indicated that Tradescantia chromosomes of cells deprived of calcium had a high rate of spontaneous breakage and were more sensitive to X-rays. Following a hypothesis that metal ions function in chromosome stability, nutritional experiments were extended to crossing over studies.—Female larvae of *Drosophila melanogaster*, heterozygous for garnet (g^2) and cut (ct^6), were raised on food containing: (1) .01 M ethylene diamine tetra-acetic acid (EDTA or versene), (2) .01 M EDTA plus .04 M potassium and (3) .15 M potassium. The most effective treatment, EDTA + K, showed in the progeny a 20 percent increase in crossing over above controls. When adult females, heterozygous for *y ct ras f*, were fed media with EDTA (.01 M to .08 M) no increase in recombination above controls was detected in their progeny. The frequency of crossing over, at least in the gametes of Drosophila larvae, can be altered chemically. The Drosophila evidence, does not permit a distinction between any of the following mechanisms of increasing crossing over: (1) higher chiasmata frequency, (2) more complete pairing, (3) less chromatid interference or (4) complications due to sister-strand crossing over.—Maize plants with the genotype *I Sh Bz Wx/C sh bz wx* were grown in quartz sand with Hoagland's solution containing optimal calcium (250 ppm Ca^{++}) or low calcium (6.25 ppm Ca^{++}). The corn plants used did not grow with less than 6 ppm calcium. Pollen was tested on detasseled plants homozygous for *C sh bz wx*. A total of 22, 109 seeds was examined. Chi square tests between treatments for each of the three regions indicated that no significant differences in crossing over existed. Pooled data gave recombination values between *C-Sh* and *Sh-Bz* which were almost identical to those reported by RHOADES ((1952), Am. Naturalist 86: 105–108). Limitations of nutritional techniques in crossing over studies are discussed. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

STORMONT, C., J. W. KENDRICK, and P. C. KENNEDY, University of California, Davis, Calif.: *A "new" syndrome of inherited lethal defects associated with abnormal gestation in Guernsey cattle.*—Data are presented on 17 affected fetuses. Gestations ranged from 256 to 526 days with a mean of 396 compared with the norm of 282. Cows carrying affected fetuses showed little sign of impending parturition. Labor which was apparently initiated only following death of the fetuses was extremely weak. The fetuses were small (average of 50 lbs.) and were easily delivered but were abnormal and immature in appearance. They exhibited varying degrees of hypotrichosis from completely hairless (excepting extremities) to almost normal. Two of the fetuses, one with fused nares and the other a pseudocyclops, classified as monsters.—In order to secure fresh tissues, three living fetuses were taken by Caesarean section. The most striking feature of the syndrome as revealed by histopathological examination was the complete absence of the adenohypophysis. Other more or less constant features of the syndrome, perhaps consequent directly and indirectly on the partial hypophyseal aplasia, are discussed.—The pedigrees of the affected fetuses traced on either or both maternal and paternal sides to a single Guernsey bull, thereby suggesting that the syndrome as a whole is transmitted as a recessive trait perhaps involving a single autosomal locus. This genetic syndrome provides unusual opportunity for research on the mechanism of parturition and the role of the adenohypophysis in fetal development.

STRAUSS, B. S., TIIU VAHARU, P. FRICKEY, and J. MATHESON, Department of Zoology, Syracuse University, Syracuse 10, New York: *Studies on the lethal effect of incorporated P^{32} and S^{35} in Neurospora crassa.*—Incorporated P^{32} and S^{35} have an inactivating effect on conidia of *Neurospora crassa*. This lethal effect is due to the transmutation of P^{32} and S^{35} rather than to the beta radiation accompanying the disintegration of these isotopes. The transmutation effects of phosphorus and of sulfur are of the same order of magnitude, a disintegration of sulfur being as likely to inactivate a Neurospora conidium as a phosphorus disintegration. Since the physical effect of the transmutation of a sulfur atom is likely to be localized in the molecule in which the sulfur occurs it is supposed that destruction of a S-containing molecule may have as lethal an effect for Neurospora as destruction of a phosphorus containing molecule. (Work performed at Brookhaven National Laboratory. Supported in part by contract AT(30-1)1138 between Syracuse University and the U. S. Atomic Energy Commission.)

STURTEVANT, F. M., Division of Biological Research, G. D. Searle & Co., Chicago 80, Ill.: *Selection for diabetic tendency in rats.*—Theorizing that glucose tolerance must be phylogenetically old and probably of polygenic determination. we attempted to select artificially for impairment of glucose tolerance, in order to develop a strain of "diabetic" rats.—Six males and six females each of Sprague-Dawley, Sherman, Wistar, and wild Norway rats were doubly-reciprocally crossed, inbreeding once to obtain a highly heterozygotic colony (Rec. GSA 22: 106). From the F_1, all rats when 12 weeks old were fasted and given an i.p. glucose tolerance test. Those with highest blood sugars were then mated twice to obtain the next generation. The 3-hour glycemias on the F_{1-6} were: 120, 186*, 177*, 162*, 196*, 210* mg.%; variances were: 796, 1742, 1133*, 987, 950, 1838* (*$P < 0.01$, compared to F_1). The mean sex ratio (m/f) and litter size of the P_3–F_5 were 1.005 and 5.5.—Although selection apparently impaired glucose tolerance, it also increased variance, resulting in a relatively stable coefficient of variation, which suggested no increase in homozygosity. Since we never observed polyuria, glycosuria, cataracts, or cachexia, we felt that the impaired glucose tolerance was probably only a quantitative change of response in which the rats remained essentially normal physiologically. Fecundity was decreased in the F_{2-4} and fertility was decreased in the F_{2-6}. This could have been due to an accidental selection of sterility factors, linkage of sterility and diabetic factors, or physiologically lowered fertility. The drastic reduction in population size necessitated discontinuance of selection.

TALLAN, IRWIN, Indiana University, Bloomington, Ind.: *Factors involved in infection by the Kappa particle in Paramecium aurelia, variety 4.*—When killer animals of stock 51 (variety 4) of *P. aurelia* are made into a concentrated brei and added to a culture of sensitive animals of the same stock (carrying the gene *K*, but lacking kappa), some sensitive animals take up kappa from the brei SONNEBORN 1947). This was demonstrated by the conversion of the sensitives into killers following slow growth to permit multiplication of the introduced kappa. This result has been confirmed for stock 51 and extended to two other stocks (47 and 169) of variety 4. Using the same concentration and volume of kappa-bearing brei, the frequency of infection of sensitives varied with the stock used as the recipient and was independent of the stock used as the source of kappa in preparing the brei. The stock differences in susceptibility to infection appear not to be due to different alleles at the *K* locus. The involvement of at least two components of the brei in infection was demonstrated by their separation into supernatant and sediment fractions by centrifugation. Recombination of the fractions resulted in much higher percentages of infection than that obtained by the use of equivalent concentrations of either fraction alone. The sediment, which should contain all the kappa, was found to retain its activity for much longer periods when stored alone (and later recombined with aged supernatant) than when stored with the supernatant. Moreover, infection has been achieved with very low concentrations of the sediment (concentrations equivalent to less than a single killer animal) in the presence of high concentrations of supernatant. (The author is a Predoctoral Fellow of the National Science Foundation. Work supported in part by an Atomic Energy Commission grant to T. M. SONNEBORN.)

TAYLOR, J. H., Columbia University, New York: *Nucleic acid synthesis during microgametogenesis in Tulbaghia violacea.*—By the use of phosphorus-32 and autoradiographs the incorporation of phosphorus into DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) was followed at stages of microgametogenesis from premeiotic interphase to mature pollen. Incorporation of P-32 into DNA of the nuclei is limited to three short periods in the 17–18 days required for development, namely, late premeiotic interphase, very early microspores, and 2-nucleate pollen grains about 6 days before the flowers open. Incorporation of P-32 into RNA occurs to a variable extent during most of the period of development, but is notably low or absent in both cytoplasm and nucleus during periods of DNA synthesis, zygotene pairing, and late stages of divisions when the chromosomes are condensed.

TONOMURA, AKIRA, and GEORGE YERGANIAN, Children's Cancer Research Foundation, Inc., Boston, Massachusetts: *Aneuploidy in the regenerating liver of the Chinese hamster.*—Chromosome complements of regenerating hepatic cells, pretreated with 0.02–0.05 mg of colchicine for 2–3 hours,

are comparable in structural detail to those noted in spermatogonia and tumor cells. Binucleation was reduced to one half the normal frequency (29%) 60–96 hours postextirpation, and then returned to normal after 144 hours. Contrastingly, treatment with triethylenemelamine (TEM) resulted in the permanent reduction of binucleated cells to half the normal value for as long as 246 hours.—Thirty of the 275 cells accurately counted for chromosome distribution were considered to have an error of ± 1 chromosome. Seventy-three percent of the cells (228) possessed exactly 22 chromosomes. However, it was further noted that 80% of these cells were true or "classic" diploids and the remainder were actually aneuploids, as determined by ideogrammatic comparisons of the 11 chromosome types. Consequently, the frequency of hepatic aneuploidy is regarded to be higher than that displayed by previous workers using rodents bearing numerous indistinguishable forms of chromosomes. Aneuploidy in normal regenerating liver is witnessed in approximately 40% of the dividing cells; whereas this condition is greatly exaggerated and involves *every* cell of tumor CH-38MC. The significance of these findings is the revelation that ⅓ of the aneuploidy resides within the peak 2n or "diploid" modality. (Supported in part by funds from the Children's Cancer Research Foundation, Inc.)

VON BORSTEL, R. C., and MARY LOU PARDUE, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *On the nature of radiation-induced dominant lethal mutations in Habrobracon and Drosophila.*—In Habrobracon and Drosophila, induction of the breakage-fusion-bridge cycle with consequent gene imbalance has been considered as the primary source of dominant lethality. Habrobracon eggs irradiated in the first meiotic metaphase show terminal deletions that result in bridges in the second meiotic anaphase and bridge-breakage during cleavage. Since the breakage-fusion-bridge cycle does not appear to become established in eggs irradiated in the first meiotic prophase, the question arises as to the cause of dominant lethality in these eggs. Examination of dead Habrobracon embryos from irradiated eggs (unfertilized, or fertilized subsequent to X-irradiation) at about the 50% lethal dose (15,000r) shows that approximately 80% have been blocked in development at the sixth or seventh cleavage and the nuclei are Feulgen negative. Thus it appears that dominant lethality in these eggs is associated with interruption of deoxyribonucleic acid synthesis. Approximately 90% of dead Drosophila embryos from irradiated sperm (ca 50% lethality; 2000r) die under similar circumstances. These resemble the specific lethality in Habrobracon in that death occurs during the early cleavage stages.—In order to test whether this action is caused by induced chromosomal deletions, the dead embryos from unirradiated triploids were examined. The chromosome complements of such embryos comprise a variety of aneuploid types. Since all eggs from Habrobracon triploids and most eggs from Drosophila triploids died during later development, it appears likely that radiation-induced dominant lethal mutations are not necessarily brought about by induction of chromosomal deficiencies. In particular, those dominant lethals associated with the radiation effect on deoxyribonucleic acid synthesis in Habrobracon are clearly not the result of chromosomal deficiencies.

WADDINGTON, C. H. (introduced by C. P. OLIVER), Institute of Animal Genetics, Edinburgh, Scotland: *The genetic basis of the assimilated bithorax phenotype.*—Abstract not available at time of printing.

WELSHONS, W. J., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *The production of wild type alleles from heterozygotes of Notch and split in Drosophila.*—Most dominant Notch mutations are visible deficiencies for at least band 3C7 of the salivary X chromosome. When such Notch deficiencies are combined with the recessive mutant split, the heterozygote has a characteristic Notch-split phenotype; Notch mutations which are not visibly deficient react with split in the same way.—Six different non-deficiency Notch mutations were chosen for study; five of these were X-ray induced and one was spontaneous. Females heterozygous for Notch and split were synthesized and crossed to split males. Since Notch is lethal in the hemizygous condition the F_1 should consist of split and Notch-split females and split males. To date, 24 not-Notch, not-split chromosomes have been recovered. At least one of these wild type alleles has been obtained from each different Notch-split heterozygote. The frequency of appearance of exceptional types varies from 1/40,000

to 1/4,500 depending on which Notch mutant is used.—Some Notch-split heterozygotes were also heterozygous for the recessive markers yellow and ruby. These crosses were *y N* + / + *spl rb* females by + *spl rb* males. From such crosses, 3 *y* + + and 2 + + *rb* chromosomes were obtained. It appears that both Notch and split can become wild type and that this unexpected event need not be associated with a recombination of the yellow and ruby markers.

WHITING, P. W., The University of Pennsylvania, Philadelphia, Pa.: ***Duplication at the R locus in Mormoniella.***—The *R* locus contains two mutable eye-color elements. Females compound for alleles recessive in different elements are double dominant wild type. A spontaneous "mutation" occurred giving males showing this double dominant effect and thus simulating diploids. Diploid males (sex homozygotes in Habrobracon) do not occur in Mormoniella. A wild type male was found in an inbred line having wild type and oyster females but only oyster males. His mother was wild type, compound for scarlet-lethal and complementary oyster, + *st l*/*oy* +. He proved to possess both of these factors. Crossed with "recessive" oyster, *oy st*, females, he sired 72 wild type daughters. These daughters and other wild type females in the pedigree, crossing with *oy st* brothers, produced females + 91, *st* 69, *oy* 160 and males + 186, *st* 154, *oy* 980. Those scarlet females which produced scarlet sons, crossing with *oy st* brothers, produced females *st* 190, *oy* 202 and males *st* 190, *oy* 646. All tested males in the pedigree were highly fertile, the wild type (+ *st l* − *oy* +) and scarlet (+ *st l* − *oy st*) presumably having the duplication, the oyster (*oy st*) lacking it. The wild type females (*oy st*/+ *st l*− *oy* +) and the scarlet females producing scarlet sons (*oy st*/+ *st l* − *oy st*) had the duplication and were of very low fertility laying many bad eggs. Oyster females (*oy st*/*oy st*) and scarlet females producing oyster sons only (+ *st l*/*oy st*) lacked the duplication and were highly fertile. The duplication does not interfere with haploid spermatogenesis but obstructs normal meiosis of the egg.

WOLFF, G. L., National Cancer Institute, Bethesda, Maryland: ***Modification of phosphatase activity of mouse lung homogenates by the $A^{v}a$ genotype and gonadectomy.***—Lung homogenates from intact 8–12 week-old yellow ($A^{v}a$) males of the Y/Ch strain had considerably *lower* alkaline phosphatase activity (pH 9.5) than those from brown (*aa*) males and females. Homogenates from $A^{v}a$ ♀♀ had definitely *higher* activity than those from *aa* animals. Gonadectomy appeared to release an inhibitory influence from the alkaline phosphatase activity in all groups except gonadectomized $A^{v}a$ ♀♀ (= $\overline{♀♀}$). In the latter, alkaline phosphatase activity was markedly *lower* than in $A^{v}a$ ♀♀. Thus, the order of activity of this group of enzymes in the different sex-genotype classes of intact animals was: $A^{v}a$ ♂♂ < *aa* ♂♂ = *aa* ♀♀ < $A^{v}a$ ♀♀. Gonadectomy, however, reversed this pattern as follows: $A^{v}a$ $\overline{♂♂}$ > *aa* $\overline{♂♂}$ = *aa* $\overline{♀♀}$ > $A^{v}a$ $\overline{♀♀}$.—There appeared to be no effects of the $A^{v}a$ genotype on the acid phosphatase activity (pH 4.6), and gonadectomy induced only a very slight rise in the activity of this group of enzymes.—The relationship of the marked effect of the $A^{v}a$ genotype on the alkaline phosphatase activity in lung homogenates to the increased lung tumor susceptibility induced by the A^{v} gene (HESTON and DERINGER 1947; MORGAN 1950) or to any of the other phenotypic effects of this gene is not clear. However, the diametrically opposite effect of gonadectomy on the alkaline phosphatase activity in $A^{v}a$ ♀♀ as compared to that in $A^{v}a$ ♂♂ and *aa* ♀♀ supports the view that the $A^{v}a$ genotype affects the endocrine system of the mouse. (U. S. Public Health Service Postdoctoral Fellow of the National Cancer Institute.)

WOLFF, SHELDON, and H. E. LUIPPOLD, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: ***Inaccuracy of measuring X-ray-induced chromosomal damage by scoring at anaphase.***—One of the most significant observations that led to the development of the target theory was the two-hit curve for X-ray-induced chromosome dicentrics and rings. There have been numerous cases in the literature, however, of a linear dose curve resulting from the scoring of anaphase bridges that ostensibly arise from the dicentrics and rings. It was decided, therefore, to examine the dose-response curves for the so-called two-hit aberrations both at anaphase and at metaphase in the same organism. Both dry and soaked seeds of barley were irradiated with varied doses of 250-kvp X-rays and then germinated. The first mitotic division was scored for chromosomal aberrations at metaphase in one half of the seeds and at anaphase in the other half. The resultant curves showed

that when metaphase figures were scored for dicentrics and rings a parabolic two-hit curve resulted, whereas the anaphase bridges increased linearly with dose. Since all the bridges were double and since the phenomenon occurred in both soaked and dry seeds, the discrepancy seems to be an artifact due to the scoring of anaphase configurations that give less information than do those at metaphase. Inasmuch as there is no constant ratio of dicentrics and rings to anaphase bridges, it should be pointed out that much caution must be exercised about correlating other radiation effects to nuclear damage as measured by anaphase bridges.

YANDERS, A. F., United States Naval Radiological Defense Laboratory, San Francisco 24, California: *An influence of aging mature sperm upon X-ray induction of sex-linked recessive lethals.*—Groups of adult *Drosophila melanogaster* (Oregon-R) males were aged in the absence of females for various periods after eclosion. Twenty-four hours prior to irradiation, each group was divided into halves, one of which was placed with an excess of females and permitted to mate ("pre-mated"); the other half remained unmated. Just before irradiation the pre-mated males were separated from the females (which were discarded), and all groups were irradiated simultaneously with 4500r of X-rays and mated immediately to Muller-5 females. Care was taken to test only progeny resulting from sperm used within twenty-four hours after irradiation.—Sex-linked recessive lethals occurred in substantially the same proportion regardless of age among all groups of pre-mated aged males and non-aged males (0–1 day old at irradiation). However, the proportion recovered from groups aged 7 days or more and not pre-mated was significantly greater. These data are in agreement with previous studies showing higher incidences of radiation-induced dominant lethals and Minutes occurring in the sperm of aged males. In addition, it is clearly indicated that some sperm storage must occur in Drosophila males, and that the increased susceptibility to irradiation is related to the age of the mature sperm rather than the age of the male at treatment.

STUDIES WITH PURPLE ADENINE MUTANTS IN NEUROSPORA CRASSA. I. STRUCTURAL AND FUNCTIONAL COMPLEXITY IN THE AD-3 REGION[1]

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Received March 5, 1956

A LARGE number of adenine-requiring strains with a purple phenotype have now been obtained for study of the problem of the reversibility of X-ray-induced mutations at the *ad-3* locus in *Neurospora crassa*. However, subsequent investigations have shown that these phenotypically similar mutants represent two distinct groups on the basis of physiological (heterokaryon) and genetic (crossing) analyses. In this paper, it will be shown that these groups represent two closely linked loci in the *ad-3* region. Studies on the mutability of X-ray-induced purple adenine mutants will be considered in a subsequent paper.

MATERIALS

New mutant strains

The 24 purple adenine mutant strains used in this investigation were obtained by the "filtration technique" of WOODWARD, DE ZEEUW, and SRB (1954) on untreated or X-irradiated macroconidia of the St. Lawrence wild type strain 74A. The isolates of these mutants used in the present investigation, with the exception of the strains with additional genetic markers, are the originals and not strains extracted from crosses to other mutant or wild type strains. The genetic background of the 24 purple adenine strains may be thus considered essentially identical.

The non-purple adenine strain, E1, was also derived from the St. Lawrence wild type strain 74A. This strain utilizes adenine but not hypoxanthine for growth (N. J. JOHNSON unpublished) and will be referred to as an adenine-specific strain.

Standard mutant strains

The other mutant strains used were: *hist-2* (histidine), C94 (HAAS, *et al.* 1952); *ad-3* (purple adenine), 35203, 38701, 38709, 45601, 68306; and *nic-2* (nicotinic acid), 43002 (BEADLE and TATUM 1945). Isolates of these mutant strains were obtained from the culture collections of PROF. N. H. GILES or DR. P. ST. LAWRENCE of Yale University.

The terminology used to refer to mutant strains is that proposed by BARRATT, NEWMEYER, PERKINS, and GARNJOBST (1954).

[1] A portion of this material was taken from a thesis presented to the Faculty of the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Report prepared under U. S. Atomic Energy Commission contract No. W-7405-eng-26. Partial support for this work was obtained under U. S. Atomic Energy Commission contract (30-1)-872 administered by N. H. GILES at Yale University.

METHODS

The medium used was the standard Neurospora minimal (BEADLE and TATUM 1945). The growth tubes used for the determination of growth rates of the heterokaryons are essentially the same as the tubes closed at one end described by RYAN *et al.* (1943). Crosses were made on synthetic crossing medium (WESTERGAARD and MITCHELL 1947) with the appropriate biochemical supplementation. Random ascospore analyses on crosses were made by the plating methods of NEWMEYER (1954). Heterokaryon tests were made with heavy conidial suspensions of the individual mutant strains. Approximately 0.03 ml of the individual conidial suspensions was placed in spots near the periphery of petri plates previously prepared with 15–20 ml of minimal medium solidified with 1.0 percent agar. Each plate contained control spots of the two strains being tested with each other, and two spots where conidia were mixed and heterokaryon formation could take place. The spots where the conidia were mixed were diametrically opposed so that when a positive heterokaryon test was obtained, the two heterokaryons grew toward each other, and did not rapidly overgrow the control spots. When heterokaryon tests were made on "leaky" strains, which grow to some extent on unsupplemented minimal medium, heterokaryon formation could be detected only by the difference in size of the colony formed on the control spot of the "leaky" strain versus the spots where conidia from both strains were mixed. All plates were incubated at 25°C and results recorded after 24–36 hours.

EXPERIMENTAL

Preliminary studies

Linkage studies with purple adenine mutant 35203 and with genetic markers known to be linked to this mutant (see BARRATT *et al.* 1954) have demonstrated that the *ad-3* locus is located 2 to 3 units to the right of the *hist-2* locus and 2 to 3 units to the left of the *nic-2* locus. Additional crosses of the 24 purple adenine strains (derived from the St. Lawrence wild type strain 74A) with the double mutant strain *hist-2 nic-2*, have shown that, in general, these purple adenine strains have linkage relationships similar to strain 35203. This evidence was interpreted as indicating that the 24 purple adenine strains and strain 35203 represented alterations of the same locus and were, therefore, presumably allelic.

However, it was not feasible to obtain more direct evidence for the allelism of these strains from intercrosses, for, as demonstrated in the original studies of MITCHELL and HOULAHAN (1946) and later studies by G. KØLMARK (unpublished) for mutants 35203, 38701, 38709, 45601, and 68306, various intercrosses and selfings of purple adenine mutants were highly sterile. While different methods were being tried to increase the numbers of viable ascospores from these crosses, attempts were made to obtain preliminary evidence for allelism from heterokaryon tests.

Heterokaryon tests with purple adenine strains derived from strain 74A

Since the 24 purple adenine strains are essentially isogenic, it seemed reasonable to assume that preliminary evidence for allelism of these mutants could be obtained if it

could be demonstrated that, under comparable experimental conditions, the individual purple adenine strains would give positive heterokaryon tests with the non-purple adenine-specific strain, E1, which has an identical genetic background, and negative tests with one or all of the other purple adenine strains.

When such tests were made by the methods described previously, positive heterokaryon tests were obtained not only between each of the purple adenine mutants and the non-purple adenine-specific strain, but also in certain combinations of the purple adenine mutants. In the initial experiment, when all 24 of the purple adenine mutants were tested against one member of this group, all the mutant combinations except three gave positive heterokaryon tests. It appeared that there were two groups of purple adenine mutants, a small group of alleles containing three of the 24 mutants (group A), and a larger group of alleles with 21 mutants (group B). Since the positive heterokaryon tests among the purple adenine mutant strains were unanticipated, an experiment was performed to confirm and extend these observations by testing all 24 purple adenine mutants for heterokaryon formation with each other in all possible combinations. Within twenty-four hours, all the group A mutants had formed heterokaryons with all the group B mutants, and no evidence was obtained for heterokaryon formation with any of the other mutant combinations. In all cases where heterokaryon formation was observed, the duplicate spots where conidia were mixed on each plate were in agreement. With one of the "leaky" purple adenine mutants, which has only a partial requirement for adenine at 25°C, the lag period was sufficient for heterokaryon formation with the three group A mutants to take place at a time when the control spots of the "leaky" mutant showed only slight growth.

On the basis of the results of these heterokaryon tests, the 24 purple adenine strains derived from the St. Lawrence wild type strain 74A can be divided into two physiologically distinct groups: group A—strains A1 through A3; group B—strains B1 through B21.

Heterokaryon tests with the original purple adenine strains

Since no evidence for heterokaryon formation between mutant 35203 and its presumed alleles was obtained in the experiments of MITCHELL and HOULAHAN (1946), backcrosses of these strains were made to obtain isolates that would form heterokaryons with biochemical mutants derived from the St. Lawrence wild type strain 74A. Originally, such isolates were available only for mutant 35203. Heterokaryon experiments on these isolates demonstrated that 35203 gave positive heterokaryon tests only with group A mutants, indicating that strain 35203 is in group B. F_1 isolates of strains 38701, 38709, 45601, and 68306 have since been used to group these mutants. Subsequent heterokaryon experiments on these strains have shown that strains 38701, 38709, and 68306 are in group A, and strain 45601, in addition to strain 35203, is in group B.

Growth rates of group A–group B heterokaryons

No evidence was obtained in the experiments on the purple adenine strains derived from 74A for marked growth rate differences in any of the group A–group B heterokaryons. All the group-A combinations with group-B mutants were similar in behav-

TABLE 1

Growth rates of selected heterokaryons between group A and group B strains as compared with the individual purple adenine strains and with wild type strain 74A. (Linear growth rates on minimal medium and minimal medium supplemented with 0.1 mg of adenine sulfate per ml)*

Strain	Medium	
	Minimal (mm/hr; 25°C)	Minimal + adenine (mm/hr; 25°C)
A1	0	3.4
A2	0	3.3
A3	0	3.2
B10	0	3.6
B10 + A1	3.5	3.6
B10 + A2	3.5	3.5
B10 + A3	3.6	3.6
74A	3.4	3.5

* Each growth rate based on the linear growth made in a 40.5-hour period in triplicate tubes.

ior, the individual heterokaryons were all established at about the same time, and all the plates were overgrown by the heterokaryotic colonies at the same rate. Since the growth rates of these heterokaryons were essentially the same, experiments were performed to compare the growth rates of selected heterokaryons with the parental wild type strain 74A, to determine whether these heterokaryons grew at wild type rate. If the group A–group B mutants were not completely complementary in the heterokaryons formed between them, then the growth rates of these heterokaryons might be less than wild type rate, and approach or equal wild type rate only by supplementation with exogenous adenine or a comparable growth factor.

By the growth tube method described previously, growth rates of individual heterokaryons between mutants A1, A2, and A3 with mutant B10 were compared on minimal medium, and minimal medium supplemented with adenine with the growth rates of the individual mutants and that of wild type. The results of this experiment are presented in table 1. None of the individual strains from either group grew on minimal medium, but heterokaryons formed with these mutants grew at rates comparable to wild type. No evidence was obtained for adenine stimulation of any of the heterokaryons, since the growth rates on minimal medium supplemented with adenine were comparable to those obtained on unsupplemented minimal medium.

Random ascospore analyses of group A × group B mutant crosses

From the evidence obtained in the heterokaryon experiments, it was apparent that purple adenine mutants did not arise from mutation of a single functional unit, but rather from mutation of two functionally distinct units. Evidence from recombination studies, discussed previously, indicated that these mutants were located between the *hist-2* and *nic-2* loci on linkage group I. Since the linkage relationships of the 24 purple adenine strains did not indicate consistent closer linkage of one group of mutants with either of the markers, it appeared possible that the functional units

might correspond to two genes so closely linked that random fluctuations in linkage values obscured the order.

If this were the case, the order of the genes with reference to the markers could be either *hist-2 ad-A ad-B nic-2* or *hist-2 ad-B ad-A nic-2*. Hence, crosses were made with appropriately marked strains to determine whether the two groups were separable by crossing over.

Preliminary attempts with corn meal agar (BEADLE and TATUM 1945) or the Westergaard-Mitchell synthetic crossing medium supplemented with low levels of adenine sulfate (0.05–0.15 mg/ml) to intercross or self purple adenine mutants were not consistent. When ascorpores were formed, they exhibited a low viability. Supplementation of the Westergaard-Mitchell synthetic medium with higher levels of adenine sulfate (0.6–1.0 mg/ml) gave large numbers of viable ascospores when intergroup crosses were made (group A × group B). Intragroup crosses (group A × group A or group B × group B) and selfings, however, did not respond to high levels of adenine or numerous other supplementations that were tried. Even when large numbers of ascopores were obtained in these crosses, under optimal crossing conditions, the viability of the ascospores ranged from three to ten percent.

Crosses of all the individual group B purple adenine strains (except strain 45601) were made, initially, with the triple mutant strain *hist-2* A2 *nic-2* to determine whether adenine-independent isolates could be obtained from such crosses, and to determine whether the origin of adenine-independent isolates was associated with crossing over between the *hist-2* and *nic-2* markers. If adenine-independent isolates occur and are associated with normal crossing over they should be almost entirely of genotypes $+++nic\text{-}2$ or $hist\text{-}2+++$ depending on the linear order of the two groups. When these crosses were analyzed, the majority gave rise to adenine-independent isolates in the limited number of ascospores analyzed in each cross (ca 6,000). Most, or all, adenine-independent isolates from any particular cross were of genotype $+++nic\text{-}2$. No isolates of genotype $hist\text{-}2+++$ were obtained in any of these crosses.

Since most of the group B mutants did give rise to adenine-independent isolates in crosses with the marked A2 strain, and since they were associated in origin with crossing over in one direction only between the linked markers, these data were interpreted as indicating (1) that the two physiological units that give rise to group A and group B purple adenine mutants in the *ad-3* region correspond to two separate, but closely linked genes, and (2) that the linear order of these genes with reference to other selected markers in this region of linkage group I is: *sex hist-2 ad-A ad-B nic-2 me*(35809) (see BARRATT *et al.* 1954).

To confirm the foregoing conclusions, and to obtain estimates of linkage between these genes, additional crosses were made by use of multiple marked strains. Since the crosses of group B strains with the doubly marked A2 strain gave adenine-independent isolates of genotype $+++nic\text{-}2$, it was anticipated that crosses of group A strains with a doubly marked 35203 (group B) strain would give rise to adenine-independent isolates of genotype $hist\text{-}2+++$. The results of more extensive analyses on intergroup crosses of these types are presented in table 2. In each cross there is a marked correlation between the origin of adenine-independent isolates with crossing over in

TABLE 2

Genotypes of adenine-independent isolates obtained from intergroup crosses of purple adenine mutants and estimates of linkage between mutants in each group

Cross	Viable ascospores tested**	No. of adenine-independent isolates	Genotypes of adenine-independent isolates				Percent recombination
			Crossovers		Noncrossovers		
			hist-2 +++	+++ *nic-2*	*hist-2*++ *nic-2*	++++	
A2 × *hist-2* 35203 *nic-2*	16,783	44	44	0	0	0	0.52 ± 0.06
35203 × *hist-2* A2 *nic-2*	38,220	130	0	128	1	1	0.68 ± 0.04
	7,668*	27	0	27	0	0	
A1 × *hist-2* 35203 *nic-2*	16,433	61	61	0	0	0	0.74 ± 0.07
A3 × *hist-2* 35203 *nic-2*	11,883	76	76	0	0	0	1.28 ± 0.10
B10 × *hist-2* A2 *nic-2*	12,840	11	0	10	1	0	0.15 ± 0.02
	21,120*	15	0	12	1	2	

* Data from a different cross of the same mutant strains.

** Viability ranged from 80–90 percent.

one direction between the closely linked markers. In the reciprocal crosses of A2 with 35203, the association of one or the other marker with the origin of adenine-independent isolates is consistent with the order of genes postulated from previous crosses.

In these crosses, as in some of the crosses discussed previously, a few adenine-independent isolates have been obtained of "noncrossover" genotypes (table 2). Each one of these exceptional types was examined genetically, since apparent adenine-independent isolates of these genotypes can originate in a number of ways. A certain fraction of those of genotype ++++ proved to be heterokaryons, which arose presumably either as pseudo-wilds (MITCHELL *et al.* 1952; PITTENGER 1954) or through heterokaryon formation between group A and group B ascospores on the plates. These have been eliminated in the tabulation. In the cross 35203 × A2, the low frequency of these exceptional types is low enough to have arisen from multiple crossover events. In the cross of B10 × 35203, however, other phenomena may be responsible. Some or all of the exceptional adenine-independent isolates from this cross could represent instances of reverse-mutation since these strains are known to be mutable. In addition, the possibility of gene conversion must be considered in view of the recent results of LINDEGREN (1955) and MITCHELL (1955a, b).

Estimates of linkage were made from the total number of adenine-independent isolates obtained in each cross. It might be postulated that some of the apparent differences in linkage between group A and group B mutants result from small chromosome rearrangements or deficiencies. However, if it is assumed that each of the genes in the *ad-3* complex extends over a short segment of the chromosome, the estimates of linkage between mutants in certain intergroup crosses can be interpreted as indicating that the individual mutant strains have arisen by mutation occurring at different sites in each gene. On this basis, if these genes are made up of closely linked, inter-dependent units that share a common function biochemically, each of which can mutate independently of one another, selected interallelic crosses might give rise to adenine-independent isolates.

Although the viability of ascospores obtained from such crosses is still very low owing to crossing difficulties, various interallelic crosses within each of the two groups of physiological alleles have been attempted by using marked strains of A2 or 35203. Preliminary analyses on four of these crosses show that adenine-independent isolates do arise. However, since the numbers of adenine-independent isolates from any particular cross have been small, and since they are of both "parental" and "crossover" genotypes, the mechanism (or mechanisms) by which adenine-independent isolates originate in specific interallelic crosses in the *ad-3* complex remains obscure.

Preliminary evidence for complexity from a serial ascospore analysis on a group A × group B cross

Although analyses of various intergroup crosses by plating methods have indicated that adenine-independent isolates do arise, this method of analysis does not permit the recovery of the presumed complementary crossover product, the double purple adenine mutant. In order to determine whether the double mutant could be recovered from an ascus in which one of the ascospore pairs was adenine-independent, serial ascospore isolations were made from the cross ++ 35203 (group B) + × *hist-2* A2 (group A) + *nic-2* (GILES and DE SERRES unpublished). Although only a portion of this analysis has been completed, some of the results are pertinent to the present analysis. In a total of 325 asci giving complete germination (at least one member of each ascospore pair germinated), five asci have been obtained containing adenine-independent ascospore pairs. In each of these asci, three pairs of ascospores are adenine requiring and the remaining pair is adenine independent. In each case, the genotype of the adenine-independent pair of ascospores is +++*nic-2*, as might be anticipated from the results obtained from ascospore platings of this same cross (table 2).

The objectives of the current analysis of these five asci are to determine whether the presence of both singles and the double mutant can be demonstrated in each ascus by genetic and biochemical techniques.

CONCLUSIONS

Previous evidence from experiments on purple adenine mutants 35203, 38701, 38709, 45601, and 68306 by MITCHELL and HOULAHAN (1946) suggested that these mutants were allelic, since only purple progeny were obtained when these mutants were crossed among themselves. On the basis of this evidence, this locus was subsequently designated the *ad-3* locus by BARRATT *et al.* (1954). Combined evidence from the present study, however, indicates that the *ad-3* locus is not unitary, as was once thought, but rather a complex consisting of phenotypically similar purple adenine mutants that fall into two physiologically distinct groups.

The results of the heterokaryon experiments on 24 purple adenine strains derived from the St. Lawrence wild type strain 74A, and the original purple adenine strains 35203, 38701, 38709, 45601, and 68306, have demonstrated that these mutants can be assigned to one of two groups on the basis of these tests. The analyses of selected heterokaryons between members of each of these groups have shown that these two

groups of mutants have mutually complementary biochemical functions. Furthermore, such heterokaryons grow on minimal medium at the same rate as the parental wild type strain 74A.

The behavior of purple adenine mutants in crosses also indicates the presence of two physiologically distinct groups. Selfings of purple adenine mutants are characteristically highly sterile under all crossing method variations that have been tried. Few ascospores are formed and usually over 90 percent of these ascospores fail to germinate. When various crosses are made among purple adenine mutants, the high sterility characteristic of the selfings is observed only when intragroup crosses are made. Intergroup crosses show the same high germination of ascospores generally found in crosses between unrelated biochemical mutants. It appears, then, that the sterility and low germination of ascospores from crosses of purple adenine mutants is indicative of the physiological identity of the parental strains.

The analysis of intergroup crosses has shown that the two physiologically distinct groups are separable by crossing over. Moreover, in such crosses, the origin of adenine-independent isolates is associated with crossing over in one direction only between closely linked markers. In these crosses, the origin of "wild type" segregants is the same as would be expected from crosses of any two closely linked genes.

Since the purple adenine mutants fall into two groups, which give positive heterokaryon tests and which give rise to adenine-independent type predominantly by crossing over in one direction between closely linked markers, the data seem best interpreted on the basis of two functionally different but closely linked genes. Since mutation of either gene gives rise to phenotypically similar purple adenine mutants, and since these genes are closely linked, it is proposed that the gene loci be designated *ad-3A* and *ad-3B*. Group A mutants, then, are at the *ad-3A* locus and group B mutants at the *ad-3B* locus.

SUMMARY

(1) Heterokaryon experiments have shown that 24 purple adenine mutants derived from the St. Lawrence wild type strain 74A fall into two physiologically distinct groups: group A—A1 through A3; group B—B1 through B21. Additional heterokaryon experiments have shown that of the five original purple adenine strains, 38701, 38709, and 68306 are in group A, and 35203 and 45601 are in group B.

(2) In general, group A and group B strains are mutually complementary in intergroup heterokaryons, and that such heterokaryons grow at wild type rate.

(3) Random ascospore analyses of intergroup crosses have demonstrated that the origin of adenine-independent isolates is associated with crossing over in one direction only between closely linked markers. The physiological groups thus correspond to two functionally different, but closely linked genes. Since mutation of both of these genes gives rise to phenotypically similar purple adenine mutants, it is proposed that the gene loci be designated *ad-3A* and *ad-3B*. Group A mutants result from mutation at the *ad-3A* locus and group B mutants from mutation at the *ad-3B* locus.

(4) The order of these loci in relation to selected markers in linkage group I is as follows: *sex hist-2 ad-3A ad-3B nic-2 al-2*.

ACKNOWLEDGMENTS

The author wishes to express his deepest appreciation to PROFESSOR NORMAN H. GILES for his guidance, criticism, and encouragement throughout the course of this study. The main part of this work was carried out during the tenure of a Wadsworth Fellowship from Yale University.

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THE RELATIVE CONSTANCY OF THE X-RAY-INDUCED MUTATION FREQUENCY OF DROSOPHILA MELANOGASTER SPERM IN INSEMINATED FEMALES[1]

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Received March 21, 1956

ALTHOUGH it has been known for many years that mutation rates were not constant within the male germ cells, being, for one thing, dependent on the age of the treated animal (OFFERMANN 1939), it remained for the crucial work of LÜNING (1952a and b) to dissect the daily changes in germ cell sensitivity in males, proving that chromosome breakability varied very definitely within short intervals with different stages of spermiogenesis and age at treatment. Among other things, it was shown that sperm X-rayed in females give a significantly higher mutation frequency than those irradiated in males within a few days before their discharge (LÜNING 1953, 1954; BONNIER and LÜNING 1953; ABRAHAMSON and TELFER 1954).

Evidence was also obtained, by MULLER, HERSKOWITZ, ABRAHAMSON and OSTER (1954), that the variation in breakability that exists among germ cells irradiated in the male can result in strong selective effects caused by dominant lethals. When the dose was increased there was an increasing selective elimination of offspring from the more mutable sperm. Thus the observed mutation frequency at a high dose but not at a low dose fell far below the frequency with which mutations had actually been induced, and the frequency-dosage relation was thereby falsified. In order that a more accurate determination of this relation might be made a project was then organized by MULLER to discover, if possible, conditions under which sperm of reasonably homogeneous and constant mutability might be obtained. It was thought that the most likely stage for this purpose would be that of spermatozoa in the inseminated female, since these were presumably in a stable, virtually mature condition, that could be prolonged for a considerable period.

In pursuit of evidence concerning this question it was decided that the present experiments should test the influence of the following conditions of aging on the observed mutation frequencies derived from sperm irradiated in females:

(1) aging versus non-aging of the sperm in females before their irradiation;
(2) aging versus non-aging of the sperm in females after their irradiation;
(3) aging versus non-aging of the males before they discharged the sperm that were to be irradiated in the females.

In addition, further data were sought on the relation between the mutation frequen-

[1] This work was supported by a grant to DR. H. J. MULLER and associates from the Atomic Energy Commission (Contract AT (11-1)-195).

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cies in sperm irradiated in the female and in those irradiated in the male within one or two days of their ejaculation.

MATERIAL AND METHODS

The stocks used in all the experiments to be described were of the following genotypes: "Multi-males", *sc*^{8}.Y/*y In49 B*; *bw*^{D}, and "Multi-females", X.Y *InEn y*; *st*. The X.Y chromosome here used had been kindly supplied by DR. NOVITSKI. These stocks had been made up by MULLER for the purpose of simultaneous detection of sex-linked recessive lethals, sex chromosome losses, and translocations (for description of the stocks and genetic technique see MULLER 1954a).

Since the *sc*^{8}.Y chromosome contains the normal allele of yellow, the male stocks are phenotypically grey bodied and brown, Bar eyed. The female stock has an X chromosome, the entire euchromatic region of which is inverted. The short arm of the Y chromosome is attached to the left end of the X, and the long arm of the Y is attached to the right end. Since this Y chromosome does not contain the *sc*^{8} attachment the females appear yellow. Originally there was no free Y chromosomes present. However, crossing over between homologous X chromosomes can produce X.Y chromosomes deficient for regions of the Y and also free partial Y chromosomes which may remain undetected in the stock.

The same X-ray machine was used throughout the course of all the experiments. Its output over a one-year period showed less than 10% variation, as determined by readings of a Victoreen dosimeter before and usually after the irradiation of the flies. A 1 mm Al filter was used and the flies, contained in perforated gelatin capsules, were irradiated by a G. E. Coolidge tube at 135 KVP, 20 ma, at a distance of 21.5 cm from the tungsten target. The dosage rate was about 220r/min.

In Experiment I, testing the effect of the aging of sperm in the females, a total of 1100 virgin "Multi-females", 24–48 hours old, forming series 61, were mated to non-virgin "Multi-males", 2–4 days old, for 24 hours, after which time the males were removed and discarded. To insure their non-virginity prior to use these males had been transferred 24 hours after hatching to fresh vials along with equal numbers of their sisters. The 1100 "Multi-females" after insemination were aged in bottles containing the standard sugar-agar medium (50 females per bottle). This sugar-agar medium was used (following the technique employed by MULLER in his experiments on the effects of aging of sperm on spontaneous mutation) to conserve sperm during aging by retarding egg laying. On this scheme the females were transferred to fresh sugar-agar medium every 5 days until the fifteenth day. At that time the females were etherized and placed into gelatin capsules for irradiation.

Two other series of inseminated females, 63 and 65, respectively, consisted of a total of 1850 virgin "Multi-females" (1–2 days old) that were mated to non-virgin "Multi-males" (3–4 days old) only 24 hours before irradiation. At the end of this 24 hours the males were removed and discarded and the females placed into gelatin capsules designating to which series they belonged. All the females of the three series were irradiated simultaneously, with 4000r of X-rays.

Immediately following the irradiation the females of series 61, containing sperm

aged 15 days prior to treatment, were placed, 50 females per vial, into freshly prepared vials containing standard culture medium and were transferred every 3 days for three successive "broods". About 750 of the females inseminated from 0 to 24 hours prior to irradiation, forming series 63, were also placed into culture vials, 50 females per vial, and transferred in the same manner as series 61. Although series 63 is relatively unaged it should be understood that some of the sperm tested in brood 3 were held for as long as 9 days subsequently to treatment. The remaining 1100 females, forming series 65, following the treatment were placed in bottles containing sugar-agar medium, 50 females per bottle, and aged for 15 days. During this 15-day period they were transferred to fresh sugar-agar medium every 5 days, and after this period they were transferred to culture vials, 50 per vial, for three 3-day broods, following a procedure identical with that employed for the other series.

The numbers of parents per bottle were here chosen so as to avoid overcrowding of offspring yet to provide enough of them to keep the food in good condition. The object of this was to minimize differences in selective effects on the developing F_1 that might otherwise, by more adversely affecting the mutants as compared with non-mutants in some series than in others, tend to make the frequencies found unrepresentative of those induced.

Another group of flies that was irradiated simultaneously with the above three was a group of 160 virgin "Multi-males", $2\frac{1}{2}$–$3\frac{1}{2}$ days old. These males were immediately afterwards mated to virgin "Multi-females", 8 pairs of parents per vial, for 24 hours, forming series 67. Then the males were removed and mated to another group of virgin females for an additional 24 hours, forming series 68. The females of series 67 and series 68 were replaced in their vials, after removal of the males, for 2 days, and were thereafter transferred through two more broods, each of 3 days duration. The reason that smaller numbers of parents were used per culture in these cases was because of their higher productivity, caused by the facts that (1) the females' germ cells had not been irradiated and (2) the mutation rate is lower for sperm irradiated in the male than in the female.

The offspring of all the five series were examined for the presence of exceptional yellow males produced through complete loss of either the X or sc^8.Y chromosome, long deletion of the X, or partial loss or mutation of the Y, involving the sc^8 attachment. These yellow males are easily distinguished from the "regular" class of gray males. The "regular" males were individually tested for translocations by backcrossing them to virgin "Multi-females". In the earlier work (ABRAHAMSON and TELFER 1954) it had been planned also to test the female offspring for recessive sex-linked lethals but after a considerable number of these tests had been carried out it was discovered that, through a rare coincidence, a preëxisting lethal had been present in the X chromosome of some of the parental males, so near to the left end as to be "covered" by the sc^8 portion of their Y. This circumstance invalidated the determinations of lethals.

Experiment II, performed at a different time, was designed to determine the difference, if any, produced by irradiating, in females, sperm derived from young males as compared with sperm derived from older males. To provide the sperm from

the young males, 1- to 2-day-old virgin "Multi-males" were mated to virgin "Multi-females" for two days. The inseminated females were thereupon irradiated with 4000r of X-rays, after which they were transferred through several 3-day broods, in bottles containing 25–30 females each. The same males as had been used for these inseminations were thereafter aged for 9 days with two consecutive groups of previously virgin females, for 4 and 5 days respectively, and then were mated to a new group of virgin "Multi-females" for 2 days. After that, the procedure followed for this older male series was the same as employed with the younger male series.

The offspring resulting from Experiment II were examined for exceptional males. In addition, it was found practicable in this experiment to carry out the test for sex-linked recessive lethals, since the preëxisting lethal was always covered by the sc^8.Y chromosome. For this purpose, the F_1 heterozygous Bar-eyed females were individually mated in culture vials with two sibling males. The F_1 males were not tested for translocations, however, in Experiment II.

RESULTS

The summarized results of Experiments I and II are shown in tables 1 and 2, respectively. In table 1 the Y translocations are not included except when they also include II–III translocation because of the possibility some of the females failed to carry an X with all the Y-fertility genes, in which case some of the F_1 males carrying a chromosome translocation would have failed to breed.

TABLE 1

Summarized frequency of yellow males and of translocations (II–III including Y-II–III) obtained following treatment with 4000r X-rays

Series	y ♂♂/total ♂♂	%y♂♂	Translocations/ cultures tested	% translocations
61 (inseminated ♀♀ aged before irrad.)	8/286	2.8 ± 0.9	21(1)*/106	19.8 ± 3.8
63 (inseminated ♀♀ not aged)	21/586	3.6 ± 0.8	60(1)*/326	18.4 ± 2.1
65 (inseminated ♀♀ aged after irrad.)	17/678	2.5 ± 0.6	52/253	20.6 ± 2.5
67 (1st day sperm from irrad. ♂♂)	49/1842	2.7 ± 0.4	74(1)*/651 (2)**	11.4 ± 1.2
68 (2nd day sperm from irrad. ♂♂)	46/2631	1.8 ± 0.3	81(2)*/1240	6.5 ± 0.7

* Those cases representing probable translocations but could not be confirmed and are not included in the calculations.

** Represent doubtful but untestable cases of translocations and excluded in calculations.

TABLE 2

Summarized frequency of yellow males and sex-linked recessive lethals in paternal X from ♀♀ inseminated by young or old males prior to irradiation with 4000r X-rays

Series	y ♂♂/total ♂♂	%y ♂♂	Lethal cultures/total cultures	% lethals
Young ♂♂	53/1393	3.8 ± 0.5	145/1073	13.5 ± 1.0
Old ♂♂	38/1192	3.2 ± 0.5	115/821	14.0 ± 1.2

DISCUSSION AND CONCLUSIONS

In Experiment I the values for translocations are more significant than those for yellow males because (1) the larger numbers of translocations obtained give them a higher statistical reliability; (2) translocations because of their comparatively small phenotypic effects, are relatively unaffected by differential viability that varies with culture conditions; and (3) translocations, on account of their frequency ordinarily varying as the 3/2 power of the effective dose, are expected to show an especially high variation of frequency with dose and so to provide an especially sensitive index of mutagenesis.

While none of the three inseminated female series show significant differences from each other an idea of the limits of variability of the aged series can be obtained by taking into account the approximate error to which the ratios of the percentages found in any of the series considered two at a time would be subject (see MULLER and JACOBS-MULLER 1925). Thus considering the series (61) aged before irradiation and the non-aged series (63) it can be expected of samples of this magnitude in only 5% of the trials that frequencies as closely in agreement as the present one, and differing in the given direction, would be obtained, if the frequency of series 61 was really 57% larger than that of series 63, or if it was really 40% smaller. In other words, taking confidence limits of 95%, the frequency of series 61 would have a range from 57% larger to 40% smaller than that of series 63. Similarly, as regards the series (65) aged before irradiation and the non-aged series (63), the range for the frequency of series 65 is found to be from 50% larger to 25% smaller than the frequency of series 63.

All three series show translocation frequencies considerably higher than that of the first day sperm (series 67), and the difference is significant, being <0.05 in the comparison of series 61 with 67 and <0.01 both in the comparison of series 63 and 65 with 67. The comparison of the translocation frequencies of series 67 (1st day sperm) and 68 (2nd day sperm) shows the former to be significantly higher than the latter, P again being <0.01. In fact, the translocation frequency is about doubled on passing from sperm treated 1–2 days before ejaculation to those treated 0–1 days before, just as it is doubled on passing from the latter to those treated after ejaculation.

The results presented indicate that the physiological conditions of aging the sperm when it is in the female, Experiment I, either before or after the irradiation, or aging males prior to insemination, Experiment II, have no appreciable effect on altering the mutation frequency and therefore demonstrate that the mutation rate in experiments performed at the same time with these different sperm is relatively constant. These physiological conditions do play an important role, however, as previous workers and the present results (series 67 and 68) have demonstrated, when the male is irradiated prior to insemination and successive ejaculates are tested.

It must be mentioned at this point, however, that a part of an investigation carried out in this laboratory by MULLER, HERSKOWITZ and OSTER (as yet unpublished) showed that translocation frequencies in the inseminated female were significantly different with the same dose of X-rays (1060r) when the experiments were performed at different times but under apparently as similar conditions as could be achieved. In one of these two experiments, utilizing approximately 1060r and 4240r, the trans-

locations appeared to follow the linear rather than 3/2 power rule in their frequency-dosage relationship, whereas this was not the case in the other experiment, which utilized a lower range of doses (265r, 1060r and 2650r). Their results at approximately 4000r are nevertheless in excellent agreement with the results herein reported. Our own (earlier obtained) results at 600r (ABRAHAMSON and TELFER 1954), 1.18% II–III type translocations, when compared with the combined results, 19.4%, herein reported and those of MULLER, HERSKOWITZ and OSTER at about 4000r do follow the expected 3/2 rule. The causes for the differences between the two results at 1060r and between the frequency-dosage relations in the experiments cited are as yet unknown. MULLER, HERSKOWITZ and OSTER are inclined to the interpretation that even the spermatozoa in the female may vary in mutability from one experiment to another, and even within the same female, the latter variation resulting in a selective effect that obscures the frequency-dosage relation.

The present data have again confirmed the phenomenon of apparently higher breakability of sperm chromosomes in the inseminated female than in sperm within a few days of their discharge from the male (LÜNING 1953; ABRAHAMSON and TELFER 1954). The reason for this phenemenon is still unknown. BONNIER and LÜNING (1953) have ruled out an influence of egg cytoplasm on the increased lethal rate in sperm chromosomes. LÜNING (1954a) has hypothesized that sperm stored in the female are more susceptible to breakage, possibly because of a different physiological environment.

If an appreciable proportion of the translocations found after irradiation of inseminated females had involved maternal chromosomes, one would expect to find a decreasing frequency of translocations when the less mature germ cells of the females were utilized in succeeding broods. This was, however, neither the case in our earlier work (ABRAHAMSON and TELFER 1954) nor in these experiments. Furthermore, in the present experiments nearly all the translocations were tested for an extra generation to determine their origin and all but one of these were found to be in paternal chromosomes. In still another experiment, utilizing the same stocks, virgin females were irradiated with 3600r X-rays. Over 1300 cultures derived from them were examined for translocations; the maternal translocation frequency in this experiment also was found to be extremely small; three cases were found, all involving the X.Y chromosome.

Still another leading hypothesis, that of differential restitution, postulated by BAKER and VON HALLE (1953), also can be considered as applicable to this discussion. This hypothesis states that the breakability of the chromosomes in the treated spermatozoa is the same in the different cases but that only restitutional and not other types of union can occur in 1st and 2nd day sperm, more of it occurring in the latter than in the former. (For a critique of this hypothesis see LÜNING 1954b). One new piece of evidence bearing on this question can be added here. Recent experiments in this laboratory by MULLER, HERSKOWITZ and OSTER (1955 and unpublished) have shown that when the inseminated females are irradiated at a higher CO_2 tension or in the presence of a different atmospheric environment, containing less oxygen, there are significantly fewer translocations than on irradiation in air. Since it has been demonstrated by MULLER, RAY-CHAUDHURI and MAKHIJANI (see MULLER 1939, 1940) that

no restitution occurs in sperm when it is stored in the female, it would appear that one would have to assume, to support the differential restitution hypothesis if applied to mature sperm stored in the female, either that restitution can occur under abnormal atmospheric conditions, or that the type of break produced under different conditions will influence the likelihood of its undergoing restitution rather than rearrangement at the time of fertilization. BAKER and EDINGTON (1952) have indeed postulated an effect of the latter type in the experiments of KAUFMANN and HOLLAENDER (1946), but it is regarded as unlikely by MULLER (1954, pp. 559–562). The differential breakage hypothesis of LÜNING is clearly the other main alternative. We believe that the unequivocal answer to this problem in Drosophila in still forthcoming.

SUMMARY

Experiments were performed to test the influence of aging on the mutation frequency of sperm irradiated in females under different conditions: 1. It was found that there was no significant difference in the II–III translocation frequencies obtained when sperm were not aged and irradiated, aged and irradiated, and irradiated then aged. 2. When sperm discharged from aged versus non-aged males were irradiated in females no significant difference was found in the sex-linked recessive lethal frequencies obtained.

Significantly higher mutation frequencies were again obtained, however, for sperm irradiated in females as compared to sperm irradiated in males. Moreover, sperm ejaculated on the second day after treatment had a translocation frequency about one half as great as those ejaculated on the first day after treatment, and the frequency of this latter group was almost one half that of sperm treated in females.

ACKNOWLEDGMENTS

The authors are indebted to DR. H. J. MULLER for his suggestion of the problem and his aid during the course of the investigation and preparation of the manuscript. It is their pleasure also to extend their thanks to all the members of the laboratory for their cooperation, especially to MRS. E. P. BARDWELL, MRS. S. BURGER and MRS. E. POYNTER for their very fine technical assistance, and to DR. I. H. HERSKOWITZ for his encouragement, suggestions and unfailing cooperation.

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AN EXCEPTION TO MUTUAL EXCLUSION OF THE CILIARY ANTIGENS IN PARAMECIUM AURELIA[1]

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Received March 26, 1956

THE system of ciliary antigens of *Paramecium aurelia* has provided abundant material for the study of the inheritance of diverse phenotypes by organisms of identical genotype. Each animal of any one stock of *P. aurelia* has the genetic potential for the expression of a series of serologically different alternative ciliary antigens (SONNEBORN 1948, 1950a; SONNEBORN and LESUER 1948; BEALE 1954). Single genes have been shown to control the serological specificities of each of the antigens. Allelic genes have been found to control the specificities of serologically corresponding serotypes of different stocks of variety 4, in all cases thus far investigated (SONNEBORN 1950a, 1951; BEALE 1954). Normally only one antigen is detectably present at any one time in a single animal. The identity of the antigen present on the cilia of an animal is referred to as its ciliary antigenic type or serotype. Serotypes can be inherited through varying numbers of vegetative and sexual reproductions. SONNEBORN and LESUER (1948) have shown that the cytoplasm plays an important role in serotype inheritance, by crossing serotypically different clones of the same stock. If no cytoplasm was exchanged the lines of descent from the exconjugants retained the serological differences of the conjugants, despite the fact that they must have become genically identical. However, if large amounts of cytoplasm are exchanged, often the lines of descent from both exconjugants of a pair have the same serotype, sometimes that of one exconjugant and sometimes that of the other.

Which serotype will be expressed is determined by the interaction of the genes controlling the specificities of the different antigens and various factors such as temperature and growth rate (SONNEBORN 1947, 1950a; BEALE 1948, 1954; SKAAR 1952; SONNEBORN, OGASAWARA and BALBINDER 1953; MARGOLIN 1955). Changes in these factors, without any change in genotype, may cause transformations from one serotype to another. During the period of transition, usually lasting only about one to two fissions, it is often possible to detect as present at the same time, both the disappearing initial serotype and the new serotype coming to expression (BEALE 1948, 1954; MARGOLIN 1955). Normally this is the only time at which two different antigens, whose specificities are controlled by nonallelic genes, are to be found detectably present at the same time in a single animal.

[1] Contribution No. 610 from the Department of Zoology, Indiana University. This work was supported by grants to T. M. SONNEBORN from the Rockefeller Foundation and the Atomic Energy Commission (contract AT(11-1)-235).

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The expression of any one serotype to the exclusion of all others potentially expressible has been called mutual exclusion (SONNEBORN 1950a; BEALE 1954). The primary basis for the exclusion is not differences in optimal conditions of culture or temperature for the different serotypes; pure cultures of different serotypes may be maintained side-by-side under the same conditions of culture. This phenomenon of mutual exclusion provides one of the most interesting aspects of the antigen system in *P. aurelia* from the point of view of developmental genetics. The present report describes an apparent exception to mutual exclusion in stock 172 of *P. aurelia*, variety 4.

NOMENCLATURE, MATERIALS AND METHODS

The serotypes are designated by capital letters to which stock numbers are prefixed since corresponding serotypes from different stocks may differ serologically. The homologous antisera are given the same designations as the serotypes against which they were obtained. Thus 172D antiserum is the homologous serum of antigenic type D of stock 172. A corresponding antiserum for a serotype is one obtained against the corresponding serotype of a different stock. Thus 172D antiserum is a corresponding antiserum for serotype D of stock 32. Small letters are used to designate the genes controlling the serological specificities of the serotypes and the stock designations are given as superscripts. For example, d^{172} designates the gene controlling the serological specificity of serotype D in stock 172.

The majority of the work described here was carried out on stock 172 (MARGOLIN 1955) of variety 4, *P. aurelia*. The use of another stock for some portions of the work will be indicated in the appropriate places. The two culture media used were a dehydrated lettuce (Difco) infusion and a dehydrated rye leaf (Cerophyl) infusion. The medium used will be indicated in the appropriate places in the description of the results. Both media were inoculated with *Aerobacter aerogenes*, incubated for 24 hours at 27°C and finally adjusted to approximately pH 6.4 before use (SONNEBORN 1950b).

The presence of a specific antigen in animals being tested is indicated by a retardation of the forward swimming movement in the appropriate antiserum. In serum of high enough concentration, following a sufficient time lapse, complete immobilization of the test animals will result. At the concentrations used for testing (SONNEBORN 1950b), the unabsorbed antisera are remarkably specific, usually having no visible effects on animals of non-homologous or non-corresponding antigenic types. A 10% Ringers solution was used as the diluent for all the sera used in these investigations.

The antisera used were obtained by following, in general, the procedures described by SONNEBORN (1950b). Four anti-D sera and 2 anti-M sera were used as well as antisera against several other antigenic types. The specific antisera used will be indicated in the appropriate places in the section describing the results.

Maximal fission rates were maintained in slide depressions by means of daily reisolations into fresh culture medium (SONNEBORN 1950b). Specific fission rates in test tubes were maintained by daily addition of appropriate quantities of culture medium (PREER 1948; SONNEBORN 1950b).

RESULTS

Simultaneous expression of two antigens, its characteristics and its maintenance

The first indication of the simultaneous expression of two antigens was noted as a decreased reactivity to 51D antiserum of serotype 172D animals. The animals were being grown in slide depressions at maximal fission rate at 31°C by means of daily reisolations into fresh Difco culture medium. Animals from the same depressions reacted somewhat weakly to 51D serum and also to both 32M and 131M antisera. The reaction to 131M antiserum, a variety 8 serum, was stronger than to 32M antiserum which had been obtained against serotype M of the variety 4 stock 32. It could be readily demonstrated that the same animals reacted to both D and the M antisera. One half of the animals from a clone consisting of the progeny of one animal produced in the course of one day were placed in 51D antiserum and the remaining animals placed in 131M antiserum. All the animals in both the sera showed a reaction by having a greatly reduced rate of swimming after 30 minutes. At the dilutions of antisera used, none of the animals in either serum was completely immobilized. Normally serotype 172D animals are immobilized within 15 minutes in such a dilution of 51D antiserum. The animals which were originally in D serum were then placed in M serum and vice versa. After 30 more minutes all the animals in both sera were completely immobilized. Thus, the conclusion that both the D and M antisera affect the same animals, which was initially indicated by the fact that both sera affected 100% of the animals in samples from the same clone, is further supported by the fact that animals showing a partial reaction to one of the sera show an additional reaction in the other serum. This suggested that both the D and the M antigens were being expressed simultaneously in the same animals.

Initially it was suspected that animals seeming to express both D and M antigens simultaneously also could at the same time express antigen J, but this proved otherwise. The DM animals also reacted to a 29J rabbit antiserum. However they did not react either to a 29J chicken antiserum or to a 51J rabbit antiserum, although no significant serological difference has been found between the J antigens of stocks 29 and 51. Moreover a pure J serotype occurs in stock 172 and these 172J animals react normally and strongly to both of the antisera (29J and 51J) to which the DM animals failed to react. It seems likely that the reaction to the original J serum was in reality a cross-reaction unique to that serum and not indicative of the simultaneous presence of the J antigen along with the D and M antigens in the animals tested. For our present purposes we may then disregard the possibility of a triple DMJ type and return to the problem of the double expression of D and M in single animals, specifically, is this to be considered as a true breakdown of the principle of mutual exclusion?

Such a breakdown would not exist on any one of three possible explanations. The most obvious of these, that the tests were made on animals which were in the process of transformation in the usual manner from serotype D to serotype M, could be eliminated immediately. Daily tests of the reisolation lines showed that the reactivity to both D and M sera could be maintained for more than 30 fissions, whereas com-

plete antigenic transformations, as has been mentioned previously, normally occur within one to two fissions in animals that are well fed. The other two explanations are based on the fact that cross-reactions are known to occur between antisera and serotypes which are neither homologous nor corresponding (SONNEBORN and BEALE 1949; BEALE 1954; MARGOLIN 1955). In other words, double reactivity could be due to either a cross-reaction of serotype 172D with M antisera or of serotype 172M with D antiserum.

The possibility that 172D animals merely cross-react to M antisera is easily ruled out. The DM animals described above react comparatively weakly to anti-D serum and also react to anti-M serum. There occur in stock 172 animals that are clearly type D only; they react strongly to anti-D serum and do not cross-react to anti-M serum at all so that the DM animals are clearly different from 172D animals. The second of the two possible cross-reactions, serotype M with D antiserum, could explain the decreased activity of D antiserum, but it is eliminated by some of the following observations.

Animals of stock 172 reactive to anti-M sera never fail to react also to anti-D sera. This of course is consistent with the interpretation that the latter reaction represents a cross-reaction rather than dual expression of two antigens. However, a cross-reaction should be constant. That is, if only a single antigen, M, were present and cross-reacting with D antiserum, the strength of the reactions to both the M and D antisera should remain the same from clone to clone and day to day. In order to discover whether such constancy exists, attempts were made to obtain the double-reacting animals under diverse conditions and to ascertain whether the reactions were identical under all conditions that yield it at all. Series of six lines, initially pure type D, were carried at maximal fission rate by daily reisolations at 35°, 31°, and 27°C. Similar sets of lines were grown at 19°C with reisolations every second day and at 14°C with reisolations every fourth day. The greater intervals between reisolations at these latter two temperatures served to compensate for the slower growth at low temperature and to provide sufficient numbers of animals for testing from each discard depression following reisolation. Half the animals remaining in each discard depression at every temperature were tested in 51D antiserum at an effective 1/200 dilution and half in 131M antiserum at an effective 1/100 dilution. The tests were examined after two hours. The results were then classified as to whether the animals were reactive to only one of the antisera or to both. In the latter case they were further classified as to whether the degree of the reaction was greater in the D serum, greater in the M serum or not significantly different for both sera. Figure 1 shows, as examples, results for two of the lines at 27°C and two of the lines at 31°C. It can readily be seen that the reactivity to the two sera varies from time to time for the same line, sometimes D serum being more effective, sometimes M serum being more effective and sometimes the two sera being equally effective. This sort of variation is definitely not consistent with the view that a cross-reaction of the pure M antigen is being detected with the D antiserum, as was suggested above. A pure M antigen would have shown a consistently similar reaction from day to day to both the corresponding M serum with which it was reacting and the D serum with which it was cross-reacting. The only simple remaining explanation for such a reaction to

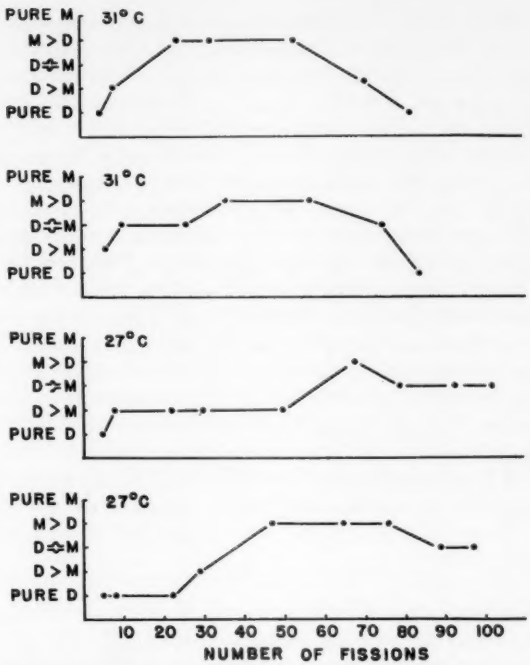

FIGURE 1.—Comparisons of the strengths of reactions to 51D and 131M antisera of two daily reisolation lines of initially 172D animals grown at 27°C and two grown at 31°C.

two different sera is that two antigens are present on the cilia at the same time and that the mutual exclusion mechanism is either not operating or its effects are being subverted in some manner. The variation in the effectiveness of the two sera supports the conclusion that the two antigens are present at the same time since DIPPELL (1953) in variety 4 and BEALE (1952, 1954) in variety 1 have found that such variations occur in heterozygotes where, with dominance being absent, two allelically controlled but serologically different antigens are expressed simultaneously.

The simultaneous expression of the D and M antigens was obtained at most temperatures tested, but under no conditions was the M antigen detected alone. The 14°C lines were discarded after approximately 35 fissions without any M antigen ever being detected, but three of the lines eventually transformed to serotype G. At 19°C only some of the lines showed a reaction to M serum and then only sporadically and weakly. At the three higher temperatures all the lines at one time or another showed strong reactions to the M antiserum. The higher the temperature the fewer fissions before all the lines expressed M. At 35°C all the lines showed a reaction to the M antiserum by the 7th fission and the reaction to M began to disappear by about the 50th fission. The disappearance of the M reaction was accompanied by a marked decrease in fission rate and 5 of the 6 lines died out shortly afterward. At

31°C the lines all reacted to M antiserum by the 10th fission although some of the lines reacted earlier. The M reaction began to disappear shortly after the 80th fission and the disappearance was again accompanied by a marked decrease in fission rate followed by death of most of the lines shortly afterward. At 27°C not all the lines were showing reactions to M antiserum until the 40th fission, although one line showed it as early as the 5th fission. All the lines continued to express M throughout the course of the experiment, lasting about 100 fissions for these lines. There was no marked decrease in the fission rate of these 27°C lines.

From the above description it seems that the appearance and maintenance of the M antigen in stock 172 is related to a high metabolic rate. The higher the temperature and the higher the fission rate, the more rapidly it comes to expression and the more readily it is maintained. Growth in depressions, when held to low fission rates of approximately one fission per day by limiting the food supply, does not bring about the expression of the M antigen. If the M antigen is already present when growth at such low fission rate is begun, it disappears. Under no conditions could the presence of the M antigen be maintained in test tube cultures, not even at maximal fission rate at 31°C.

A second set of daily reisolation lines was grown at 31°C and tested periodically in a series of dilutions of both the D and M antisera to determine the immobilization titres in these two sera. The immobilization titre is defined as the greatest dilution

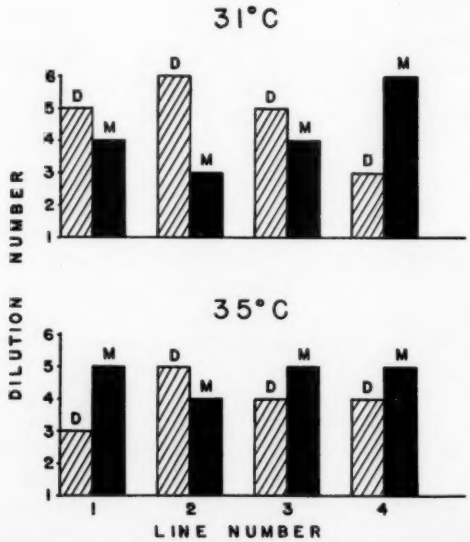

FIGURE 2.—Immobilization titres in 51D and 131M antisera of 4 daily reisolation lines at 35°C and 4 at 31°C, showing the negative correlation of the variation in titre in these two sera. Dilutions corresponding to those represented by the numbers 1 to 6 are 1/12.5, 1/25, 1/50, etc. to 1/400, respectively, each being half the concentration of the preceding one.

which causes immobilization in two hours (SONNEBORN 1950b). Comparisons of the titres in the two sera do not indicate quantitative ratios of the two antigens since the two sera are not necessarily equivalent in their ability to immobilize. For example, a higher titre in M serum does not necessarily mean that there is more M antigen present than D antigen. It may merely mean that the M serum is a stronger serum than the D serum. However, comparison of the variations in titre of the M serum with variation in titre of the D serum serves to indicate relative variations in the quantities of the two antigens on the animals. There is found a negative correlation between the variations in the titres in the two sera: the higher the titre in the D antiserum, the lower the titre in the M antiserum. When the titre in M antiserum rises the titre in D antiserum falls. This is shown by figure 2 which compares the immobilization titres in the D and M sera. The values in figure 2 represent single observations. The differences between the lines at each temperature do not remain constant since the D and M titres of each line vary with time. The variations in titre most likely indicate variations in the quantities of each of the antigens, as suggested above. If the simultaneous expression of the two antigens is due to a breakdown of the mechanism of mutual exclusion then the negative correlation may indicate some sort of competitive relationship between the antigens. Such competition could either be for the antigen sites on the cilia or for some common substrate or precursor which is in limited supply.

Attempts to establish simultaneous double expression of serotypes other than D and M

If one attempts to conceive of a relationship between two serotypes which might be conducive to their simultaneous expression, similarity in conditions for stability is one which immediately comes to mind. It is not possible to determine whether or not such a relationship exists in the case of D and M since the conditions for maintenance of a pure M serotype have never been found. However, to determine if other double types could be established when such a relationship is known to exist, the following two experiments were carried out.

For the first experiment serotypes 172B and 172G were chosen because both were found to be very stable under the same conditions of culture. Lines of these two types were grown for more than 50 fissions in daily reisolations at 27°C; they very rarely transformed to any other serotypes during this period of growth at maximal fission rate. But each line also remained pure for the initial type, either B or G, never showing simultaneous expression of both. There still remained the possibility that if animals, which were already expressing both types because they were in the midst of a transformation from one to the other, were placed under these conditions, the double type might be maintained. It had been found previously that B animals transformed primarily to G at 14°C, following exposure to immobilizing antiserum (MARGOLIN 1955). Serotype B animals were exposed to antiserum and washed as previously described (MARGOLIN 1955) and then isolated into fresh Difco medium and placed at 14°C. Every 4 hours during the next 12 hours groups of six isolations were transferred to 27°C. Two additional groups were later transferred at eight hour intervals. The transferred lines were then grown at maximal fission rate at 27°C by daily reisolations. Each day's discards were tested for the double expression of B and

G. No such double expression was encountered in any of the lines. Some lines remained B, some transformed to G and some to other serotypes.

A second experiment which attempted to determine if similar conditions for stability could be a basis for simultaneous expression of two serotypes made use of cytoplasmic exchange to initiate a mixed cytoplasm. As mentioned previously, it had been found that the cytoplasm plays a part in determining which serotype is expressed. It was felt that an exchange of small amounts of cytoplasm between two serotypes having the same conditions for stability, followed by growth under these conditions might initiate and maintain a double expression. Serotypes 172B and 172D were chosen as the two types. Type B, as mentioned above, is quite stable in daily reisolation at 27°C. Type D is also stable under these conditions in the sense that at least some D antigen continues to be expressed. The M antigen normally also appears along with D under these conditions indicating that D is at least amenable to double expression, and bringing up the possibility of obtaining a triple type, BDM.

The cytoplasms of D and B animals were mixed by obtaining an exchange of cytoplasm during conjugation. The process has been described by SONNEBORN (1950b). The length of delay in complete separation of conjugants and the broadness of cytoplasmic bridges which may develop between the separating conjugants are taken as indices of the amounts of cytoplasm exchanged. In this experiment no broad bridges were formed and the delays in separation ranged from 5 to 44 minutes among 12 conjugating pairs. Under these conditions only comparatively small amounts of cytoplasm can be expected to have been exchanged. All the exconjugants were then carried as daily reisolation lines at 27°C for approximately 18 fissions. Each day's discards were all tested with B, D and M antisera. In no case was the B antigen found expressed simultaneously with any other antigen. It would appear from the results of this and the preceding experiment that similarity in conditions for stability alone is not a sufficient basis for the simultaneous expression of two antigens.

There remains one additional type of relationship known to exist between some serotypes which might conceivably serve as a basis for the maintenance of double antigenic expression. If two serotypes which readily and preferentially transform to each other could be maintained in a state of constant, rapid transformation back and forth, the detectable results would be the simultaneous expression of the two antigens. This sort of an explanation for double expression has in its favor that it does not require a breakdown of the mutual exclusion mechanism. SONNEBORN (unpublished) had found that in stock 131 of variety 8 the above described sort of relationship existed between serotypes E and H. At a low fission rate type E transformed readily to type H, whereas at a high fission rate H transformed to E, all at 27°C. To determine whether or not at some intermediate fission rate a state of continuous transformation from one serotype to the other could be maintained a series of test tube cultures, initially pure for 131H, were grown at various fission rates. Average fission rates of $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, 2 and 3 fissions per day were maintained by daily adding appropriate quantities of Difco culture medium and duplicate cultures were grown at each rate.

After three weeks of growth one sample from each tube was tested with E antiserum and a second sample with H antiserum. The $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ fissions per day tubes were found to have remained 98% or more pure for type H. About half the non-H animals were type E and the remainder were not identified as to serotype. The remaining tubes were mixtures of animals which were primarily of types E and H. The higher the fission rate, the higher the percentage of type E animals found, ranging from an average of 9% of type E in the 1 fission per day tubes to an average of 55% of type E in the 3 fissions per day tubes. In all cases the percentage of E animals from a tube plus the percentage of H animals from that tube always added up to approximately 100%, when allowance was made for a sampling error and a small percentage of animals which were neither E nor H. This would indicate that E and H were not being expressed simultaneously in the same animals. Such a double expression would have been expected if a state of continuous back and forth transformation between E and H existed. Apparently a readiness for two serotypes to transform to each other preferentially is not alone sufficient to support such a state.

Genetic analysis

The experiments described in the previous section indicate that the two sorts of relationships which were considered, similarity of conditions of stability for two serotypes and preferential transformation back and forth between two serotypes, do not support simultaneous expression of two antigens. This suggests that some special relationship may exist between 172D and 172M which has not been recognized. Since the relationship itself is not at present discernible the next approach would be to attempt to determine at least on what level it exists. That is, whether it occurs only for the specific D and M serotypes of stock 172, or for D and M serotypes of any stock or for any serotypes which have the necessary proper characteristics. The relationship can be divided into two parts; the role of the M serotype and the role of the D serotype. The evidence suggests that some specific characteristic of the M serotype of stock 172 is involved. It is not a general characteristic of M antigens in *P. aurelia* as indicated by the fact that serotype M of stock 32, variety 4, always appears as a single antigenic type. Serotype 172M not only differs from all the serotypes except D by showing the capacity for dual expression, but it even differs from D in never showing the capacity for single expression under any of the conditions used. There thus seems to be something very special and, thus far, unique about antigen 172M that makes double expression possible.

But there is more to the story than this, for 172M is expressed only with D, not with any other serotype,—not even with any serotype that is stable under conditions in which M is expressed. Thus when serotypes 172B or 172G are grown at maximal fission rate at 27°C serotype M does not come to expression. However if 172D is the initial type under these conditions type M does come to expression. The question then arises as to whether the ability to be expressed simultaneously with the 172M antigen is a general characteristic of all the D antigens or if it is a unique characteristic of the 172D serotype. Stock 32 has both D and M serotypes but they have never been found expressed simultaneously. Type 32M perhaps does not have the proper

characteristics to permit such double expression. Would the 32D serotype come to double expression with 172M if the d^{32} gene were substituted for th d^{172} gene in the stock 172 genome?

Fortunately serotype 172D is serologically distinguishable from serotype 32D, so that the above question can be answered. The sera used to distinguish these two serotypes from each other were 138D and 32D. The immobilization titres of each of the serotypes were determined in each of the antisera using the methods described by SONNEBORN (1950b). It was found that 138D antiserum had a clearly higher titre against 172D animals (immobilization titre: 1/200 dilution) than against 32D animals (immobilization titre: 1/50 dilution), whereas the titre of 32D antiserum was higher against 32D animals (immobilization titre: 1/200 dilution) than against 172D animals (immobilization titre: 1/50 dilution). Dilutions of these two sera were chosen which readily distinguished the two antigens from each other by the differences in the strengths of their reactions in each of the sera, following exposure for two hours. In this case, at the dilutions of sera used, the heterozygote could not be distinguished from the 32D homozygote. This does not necessarily indicate dominance of the d^{32} gene. DIPPEL (1953) has found in a similar case in variety 4 that the heterozygote could be distinguished from the homozygote which it resembled if enough different sera were tried. All the work described below was carried out in Cerophyl culture medium.

Eight backcrosses to stock 172 were made so as to approach isogenicity. The F_2 obtained by autogamy from the final backcross gave a ratio of 42:41 for the 32D antigen and 172D antigen, respectively (four F_2 lines died before testing). This was clearly a 1:1 ratio, indicating allelism of the d^{32} and d^{172} genes. The numbers obtained are too small to distinguish between alleles and closely linked genes, but no cases of serologically corresponding serotypes have ever been found to be non-allelic in variety 4 (SONNEBORN 1950a, 1951, unpublished; BEALE 1954).

One of the F_2 lines, homozygous for d^{32}, obtained from the eighth backcross described above was crossed once again to 172D. The exconjugants, which were then heterozygous for the two alleles for the D serotype, were grown in daily reisolation lines at maximal fission rate at 31°C. Controls consisted of crossing the 172D animals used in the above cross to another clone of 172D animals of the complementary mating type. The exconjugants from this control cross, which were homozygous for the d^{172} gene, were grown under the same conditions as the experimental heterozygotes. Ten exconjugants of each sort were cultured as described and animals in discard depressions were periodically tested with 32D, 172D and 131M antisera. The 172D antiserum was used in place of 138D antiserum. These two sera act similarly in distinguishing between the two D serotypes. The M antigen was detected in both the experimental and the controls and as expected it was always accompanied by the presence of some D antigen. The presence of a single dose of the d^{32} gene did not prevent the simultaneous expression of the D and M antigens but it did have an effect. All the homozygous 172D lines manifested some M antigen before even one of the heterozygotes was manifesting it. In fact one of the heterozygote lines never showed any M antigen during the 54 fissions it was kept. Another effect which was noted was that the quantity of M antigen, as indicated by the strength of the reaction

in the M antiserum, never became as great in the strongest M reacting heterozygotes as it did in the strongest M reacting lines which were homozygous for the d^{172} gene. The single d^{32} gene, while not completely preventing the double expression of the D and M antigens, clearly had the effect of retarding and diminishing the expression of the M antigen.

The next step consisted of testing the effect of the homozygous d^{32} condition. An F_2 was obtained by autogamy from one of the heterozygotic lines used for the experiment above. Five of these F_2's, homozygous for d^{32} and five homozygous for d^{172} were tested for the presence of the M antigen. All were found to be expressing only the D serotype. Three subcultures from each of these lines were then grown at maximal fission rate in daily reisolations at 31°C. The 15 reisolation lines homozygous for d^{32} and the 15 homozygous for d^{172} were tested periodically with 32D, 172D and 131M antiserum. The same general effect of the presence of 32D was found as had been found in the heterozygotes. The expression of the M antigen was retarded but not always completely suppressed. The degree of retardation of development of the M antigen was not significantly greater in the presence of the double dose of the d^{32} gene than in the heterozygotes containing only a single dose of the gene.

In the two experiments described above the D antigen which was detected when the M antigen was present in the lines having d^{32} genes, always reacted with the two D antisera used in the same way that the pure 32D serotype reacted. That is, the reaction was distinctly stronger in 32D antiserum than in 172D antiserum. The reverse was always true in the lines which were homozygous for the d^{172} gene. This is shown clearly in table 1. The 32M antigen is readily distinguishable from the 172M antigen using 32M and 131M antisera. Serotype 32M reacts more strongly than serotype 172M in the 32M antiserum and the reverse is true for 131M antiserum.

TABLE 1

Reactions to 131M, 32D and 172D antisera of samples of eight animals exposed for two hours, showing that the 32D and 172D antigens can be distinguished even when being expressed simultaneously with 172M

Lines tested		Antisera used and effective dilutions		
		131M, 1/50	32D, 1/100	172D, 1/100
I	1	+++	++	+
	2	++	+++	++
II	1	+++	−	++
	2	++	−	+++
III	32D	−	+++	++
	172D	−	+	+++

− no visible effect of antiserum.

+ least definitely perceptible retardation of movement.

++ very sluggish movement.

+++ complete immobilization.

I Two lines homozygous for the d^{32} gene and approximately isogenic with stock 172. Grown in daily reisolations at 31°C.

II Two lines homozygous for the d^{172} gene and grown in daily reisolations at 31°C.

III Samples from stock tube cultures of 172D and 32D grown at one fission per day at 27°C.

The M antigen which was expressed in the lines containing the d^{32} genes was tested in appropriate dilutions of these two sera and could always be identified as 172M. This was, of course, to be expected in approximately isogenic lines.

Since 32M and 172M were readily distinguishable an attempt was made to create a stock containing the m^{32} gene but essentially isogenic with stock 172. Great difficulty was encountered following the initial cross between stocks 32 and 172. Serotype 32M was found to be extremely unstable in the hybrid under all conditions which were tried. No means was discovered which would cause 32M to come to expression in the F_2 lines obtained by autogamy from this first cross. The lines were then grown for many months in tubes at one fission per day at 27°C and tested periodically for the presence of 32M animals. Finally one such line was discovered and used for the first backcross to stock 172. The same difficulty with 32M was encountered in this cross and the same means used to overcome it. A total of three backcrosses was finally obtained by this method. Fifteen F_2 lines obtained by autogamy from the third backcross, all expressing the 172D serotype, were grown at maximal fission rate by daily reisolations at 31°C and periodically tested for simultaneous expression of the 32M and 172D antigens. All the lines were grown for approximately 40 fissions under these conditions but in no case was such double antigenic expression encountered. One line transformed to 32M after about 25 fissions and continued to express the M antigen for the remainder of the experiment without exhibiting any detectable trace of the D antigen. The 32M antigen does not appear to be compatible with double expression even in the presence of the d^{172} gene and what was primarily a 172 genetic background. This confirms the conclusion, previously mentioned, that the capability for double antigenic expression was a specific characteristic of the 172M serotype and not a generic characteristic of the variety 4 M serotypes.

DISCUSSION

The above results demonstrate clearly that under appropriate conditions two antigens controlled by non-allelic genes can be expressed simultaneously and this dual expression is maintained as long as the conditions remain the same. This immediately raises the question as to whether or not this represents an actual lack of functioning on the part of the mechanism which normally maintains mutual exclusion of such antigens. The only simple explanation not requiring such a lack of functioning is that a state of continuously unstable transformation is being maintained between serotypes D and M. That is, serotype D transforms to serotype M which immediately transforms back to D and so on. Evidence presented in the results section indicated that a readiness of two antigens to transform to each other preferentially is not alone sufficient to maintain such a state. However, it is possible to visualize additional relationships between two serotypes which might be expected to sustain it.

For example, two antigens exhibiting the following relationships could be maintained in a state of continuous transformation under the proper conditions. The two antigenic types, as mentioned before, would have to show a very strong preference to transform to each other rather than to any of the other potential serotypes. In addition they would have to be mutually exclusive on the basis of the conditions of culture which allow their expression. That is, two serotypes, each stable in different

portions of a range of conditions of culture and each completely unstable under the conditions favoring the other. It would be necessary that there be no zone of overlapping of ranges of stability; the stability ranges must be contiguous and mutually exclusive. If animals expressing one of the two serotypes in question were then grown under conditions which approximated the boundary dividing the stability ranges of the two serotypes, slight fluctuations would cause transformations back and forth as the conditions shifted from one side of the boundary to the other. It is interesting to note that the conditions necessary for the origin and maintenance of the DM double type, maximal growth rate in the continued presence of excess food, are the very conditions which can be maintained most constantly, subject only to the minor fluctuations such as are hypothesized above. Furthermore, the M antigen is never expressed under the same conditions as the D antigen except in the very narrow range of conditions where they are expressed as a double type. The assumption, on the basis of the above hypothesis, would then be that, in the medium used, maximal growth rate just reaches a threshold value above which the M antigen can be expressed but not the D antigen. Below this threshold the D antigen can be expressed but not the M antigen. Assuming further that D and M transform to each other preferentially, the result of minor fluctuations above and below the threshold would be a continuous series of transformations between D and M.

The above explanation can also account for the retarding effect, previously mentioned, of 32D on the expression of 172M. SONNEBORN and BALBINDER (unpublished) have obtained evidence indicating that the genes for the specificities of all the serotypes in a genome interact in determining the relationship of any one serotype to environmental conditions, with respect to both stability and origin. Therefore the presence of the d^{32} gene may have shifted upward the threshold value needed for the expression of M. Fewer peaks in fluctuation would be sufficiently great to cross this higher threshold so that D would transform to M less often and the M expression would be of shorter duration and retarded. Thus by means of a formal explanation the simultaneous expression of the D and M antigens and the characteristics of this double expression can be accounted for without resorting to non-functioning of the mutual exclusion mechanism; but the latter possibility is not thereby excluded.

One somewhat inconclusive fact points to the actual breakdown of the mutual exclusion mechanism in this case. The mechanism is known to breakdown regularly with respect to allelic serotypes: a heterozygote expresses both allelic serotypes (BEALE 1952, 1954; DIPPELL 1953; MARGOLIN 1955). In a different sense alleles are also involved in the DM case. Double expression with M occurs when either of two d alleles are present, d^{172} and d^{32}. On the other hand, replacement of m^{172} by m^{32} results in failure to obtain DM, as does also the combination $d^{32}m^{32}$. The argument therefore is rather weak, but nevertheless it must be kept in mind.

In sum, both alternative explanations, repeated transformations and breakdown of the mechanism of mutual exclusion, are equally compatible with the observations. Actual criteria that would differentiate the two explanations operationally are lacking. Both would give exactly the same detectable results, namely, the simultaneous presence of both D and M antigens.

Here, however, a distinction must be made between mutual exclusion as a mech-

anism and mutual exclusion as an observation. The two explanations offered above attempt to interpret the observed breakdown of the mutual exclusion phenomenon from the point of view of the mechanism by which it is normally maintained. The observation of mutual exclusion refers simply to the fact that usually only one ciliary antigen can be detected in any animal at any one time. Two sorts of exceptions to the observation have previously been known: one is the case of allelic serotypes in a heterozygote, mentioned above; the other is the transient state of transformation, narrowly localized in time, between two relatively stable and different single serotypes. The present DM case is a third sort of exception to the observation of mutual exclusion: a persistent condition in which two non-allelic serotypes are continually expressed.

SUMMARY

Contrary to the usual condition of mutual exclusion existing among non-allelic ciliary antigens of *P. aurelia*, stock 172, variety 4 is regularly capable of expressing simultaneously two ciliary antigens, D and M. This dual expression can be maintained through many reproductive cycles when the animals are grown at maximal fission rate by means of daily reisolations at 35°, 31° and 27°C. Antigenic type D is often expressed under different conditions as a single antigen, in the normal manner, but the M antigen when detectably present is always accompanied by the D antigen. In any one line of descent expressing DM the ratios of the quantities of the two antigens vary from time to time. There is a negative correlation in the variations in quantity of the two antigens, an increase in one usually being correlated with a decrease in the other. This suggests a competitive relationship of some sort.

Attempts to initiate similar dual expression of other antigens on the basis of similarity in conditions for stability or a readiness for preferential transformation to each other of two antigenic types failed. The capacity for dual expression appears to be due to some unique quality of the 172M antigenic type. The M antigenic type of stock 32, variety 4, does not show this capacity either in the stock 32 genome or when the m^{32} gene is substituted for the m^{172} gene in a genome that was primarily that of stock 172. However, when the d^{32} gene is substituted for the d^{172} gene in a genome which is approximately isogenic with stock 172, the 32D antigen does show a capacity for dual expression with 172M, although the expression of M is somewhat retarded when compared to that which is found with the 172D antigen.

The observations can be explained as due to either a non-functioning of the mutual exclusion mechanism or a state of continuously unstable transformations between the D and M antigenic types. Operationally these two explanations are identical since they both lead to the continuous simultaneous expression of two non-allelic antigens. The persistence of the DM state and the fact that it involves non-allelic antigens, together distinguish this exception from the two previously known classes of exceptions to mutual exclusion.

ACKNOWLEDGMENT

The author is most grateful to DR. T. M. SONNEBORN for his invaluable encouragement and criticisms throughout the course of this work.

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THE HYBRID-SUBSTANCE OF THE ERYTHROCYTES OF THE HYBRIDS BETWEEN COLUMBA LIVIA AND STREPTOPELIA RISORIA[1]

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Received March 26, 1956

IT has been thought that antigens of the red blood cells are more or less direct products of their causative genes (IRWIN 1939; IRWIN and COLE 1936a; HALDANE 1937). An exception is the "hybrid-substance" which seemingly consists of one or more antigenic specificities of the cells of hybrids between certain species of birds which are not possessed by the cells of either parental species (IRWIN 1932; IRWIN and COLE 1936a; IRWIN and CUMLEY 1945; MCGIBBON 1944, 1945). The erythrocytes of the hybrids between the common domestic pigeon, *Columba livia*, and the ring neck dove, *Streptopelia risoria*, possess such a hybrid-substance (IRWIN and COLE 1936b). The segregation of this hybrid-substance in a unique backcross family and the presence or absence of related antigenic specificities in other species and certain other species hybrids of Columbidae are presented in this paper.

MATERIALS AND METHODS

Briefly, the procedure used to demonstrate the hybrid-substance was to produce antisera in rabbits to cells of the F_1 hybrids, and then to absorb the resulting antisera at an appropriate dilution (usually 1:60) with the pooled cells of many individuals (about 10–30 each) of both parental species. If possible, the cells of the actual parents of the hybrids were used in these absorptions and as controls in the agglutination tests in order to eliminate the possibility that individual differences within either parental species might be a complicating factor in the detection of the hybrid-substance. The agglutination of the cells of the F_1, and a lack of agglutination of the cells of the parental species with the resulting "reagent" were evidence for a new specificity—the "hybrid-substance"—possessed by the F_1 cells. These tests were usually performed with the reagent at the absorbing dilution (1:60), and with the dilutions serially doubled, if the titer was determined. This reagent could be fractionated by further absorptions with cells of other species or species-hybrids which possessed serological specificities that were related to the hybrid-substance. Additional details of pertinent immunological procedures such as absorption ratios of cells to serum, agglutination tests, etc., may be found elsewhere (CUMLEY and IRWIN 1941a, 1941b, 1942; IRWIN and COLE 1936a; MILLER 1953).

Most of the reagents or test fluids used in the present study were prepared from

[1] Paper No. 594 from the Department of Genetics, University of Wisconsin. This investigation was supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, and by a research grant (G-3884 to M. R. IRWIN) from the Department of Health, Education and Welfare of the National Institutes of Health, Public Health Service.

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four antisera (493F4, 495F6, 480F4 and 491F2) produced in rabbits against the cells of hybrids between *livia* and *risoria*. The titers of the reagents for the hybrid-substance prepared from these four antisera varied from three to eight doubling dilutions beginning at 1:60. Antisera against the cells of the parental species, and against cells from *C/C* and *C/C′* backcross hybrids of *C. guinea* × *C. livia* (MILLER and BRYAN 1953) were also used.

Various backcross hybrids from the cross of *C. guinea*, the triangular spotted pigeon of Africa, with *livia* were available for tests. IRWIN, COLE and GORDON (1936) demonstrated the species-specific antigens A, B, C and E of *guinea* in contrast to *livia*; MILLER and BRYAN (1953) disclosed the presence of the species-specific antigens of *livia*, A′, B′, C′ and E′ which are antithetical and presumably produced by genes allelic to those producing the respective antigens of *guinea*. The cells of backcross birds carrying other species-specific characters specific to *S. chinensis* (d-1, d-4 and d-11) and *S. senegalensis* (s-8 and s-10) respectively, in contrast to *risoria*—were included in some of these tests (see IRWIN 1953 for leading references). Other characters specific to *S. orientalis* (or-4 and or-8) and *S. tranquebarica* (hu-4 and hu-8) have been obtained in unit form following crosses and backcrosses of each species to *risoria* (IRWIN unpublished data).

The species and F_1 hybrids used were as follows: *Streptopelia bitorquata* (also known as *dussumieri*), Philippine turtle dove; *S. capicola*, cape turtle dove; *S. chinensis*, pearlneck or laceneck dove; *S. orientalis*, Oriental, Asiatic, or Chinese turtle dove; *S. risoria*, ring neck, barbary, domestic, fawn, blond or ring dove; *S. semitorquata*, African turtle dove; *S. senegalensis*, senegal or palm turtle dove; *S. tranquebarica* (also known as *humilis*), dwarf or red backed turtle dove; *S. turtur*, European turtle dove; *Columba fasciata*, bandtail pigeon; *C. flavirostris*, yellow billed pigeon; *C. guinea*, triangular spotted or speckled pigeon; *C. livia*, common, domestic, or rock pigeon; *C. maculosa*, spotted pigeon, *C. picazuro*; *C. rufina*, rufous pigeon; *C. palumbus*, European wood pigeon; *Chalcophaps indica*, green wing dove; *Gallicolumba luzonica*, bleeding heart pigeon; *Geopelia humeralis*, bar-shouldered dove; *L. cassini*, cassin's dove; *Leptotila jamaicensis*, violet dove; *Nesopelia galapagoensis*, Galapagos dove; *Ocyphaps lophotes*, Australian crested dove; *Phaps chalcoptera*, bronze wing pigeon; *Phaps elegans*, brush bronze wing pigeon; *Zenaida aurita*, Martinique dove; *Zenaida asiatica*, white wing dove; *Zenaidura auriculata*, bronze neck dove; *Zenaidura graysoni*, Grayson's dove; *Zenaidura macroura*, mourning dove; *Caloenas nicobarica*, Nicobar pigeon; *Ducula spilorrhoa*, white fruit pigeon; *Goura cristata*, Goura crowned pigeon; F_1-*bitorquata/risoria*; F_1-*chalcoptera/elegans*; F_1-*chinensis/risoria*; F_1-*chinensis/orientalis*; F_1-*livia/risoria*; F_1-*orientalis/risoria*; F_1-*semitorquata/risoria*; F_1-*semitorquata/orientalis*; F_1-*senegalensis/risoria*; F_1-*senegalensis/capicola*; F_1-*tranquebarica/risoria*; F_1-*asiatica/risoria*; F_1-*macroura/risoria*; F_1-*fasciata/livia*; and F_1-*C. oenas/livia*. The number of individuals used within each species varied greatly. Only one to a half dozen were available in those species difficult to obtain (perhaps half of the above list).

MR. J. P. TELFORD of Madison, Wisconsin, kindly allowed blood samples to be taken from his unique family of *livia-risoria* hybrids and backcross hybrids. MR. KARL PLATH, curator of birds, Chicago Zoological Park, kindly permitted the author

to obtain blood samples from *nicobarica*, *spilorrhoa*, *lophotes* and *guinea*; and MR. R. MARLIN PERKINS, director, Lincoln Park Zoological Gardens, Chicago, Illinois, permitted the author to obtain a blood sample from *cristata*.

RESULTS

Segregation of the hybrid-substance with a species-specific antigen

Hybrids between the domestic pigeon, *Columba livia*, and the ring neck dove, *Streptopelia risoria*, are usually sterile (COLE and HOLLANDER 1950). Nevertheless, MR. TELFORD raised a male F_1-*livia/risoria* which in a backcross to *risoria* produced eight offspring which lived to maturity. One of these backcross birds produced a squab in mating to *risoria*. The F_1 male, in addition to possessing the hybrid-substance, had as expected, the species-specific characters (A′, B′, C′, and E′) of *livia* in contrast to *guinea* (MILLER and BRYAN 1953). The cells of *risoria* lack the A′, C′ and E′ of *livia*, and the "B′-like" specificity which they possess was easily distinguished from the B′ of *livia* (MILLER 1953). Therefore, the inheritance of the hybrid-substance and the species-specific characters of *livia* could be followed in these eight backcross and the second backcross offspring.

The data in table 1 show the segregation in the backcross offspring of the hybrid-substance and the species-specific characters of *livia*. The cells of four (Nos. 2, 6, 8 and 9) backcross birds were strongly agglutinated by any of the four reagents

TABLE 1

Segregation in backcross offspring of the hybrid-substance and of characters specific to Columba livia *in contrast to* Streptopelia risoria

Test cells	Presence or absence of agglutination of test cells with reagents for:							
	(species-specific substances of *livia*)				hybrid-substance of F_1-*livia/risoria* from antiserum			
	A′	B′	C′	E′	493F4	495F6	480F4	491F2
risoria	0	0	0	0	0	0	0	0
livia	+	+	+	+	0	0	0	0
F_1-*livia/risoria*	+	+	+	+	+	+	+	+
First backcross offspring								
1	+	+	0	+	0	0	0	0
2*	+	+	+	+	+	+	+	+
3	+	+	0	+	0	0	0	0
4	0	+	0	+	0	0	0	0
5	+	+	0	+	0	0	0	0
6	+	+	+	+	+	+	+	+
7	+	+	0	+	0	0	0	0
8	0	0	+	0	+	+	+	+
Second backcross offspring								
9	+	+	+	0	+	+	+	+

* Male parent of No. 9 in a backcross to *risoria*.

(from antisera 493F4, 495F6, 480F4, or 491F2) for the hybrid-substance as listed in table 1. The cells of all (over 40) F_1's tested have always been strongly agglutinated by such reagents. The cells of five of the backcross offspring (Nos. 1, 3, 4, 5 and 7) and, of course, *livia* and *risoria*, including the actual *risoria* parent, did not react with these reagents.

Each of the antigenic characters specific to *livia* segregated in these backcross offspring, as expected if they were genetically determined. Thus, there were seven backcross birds with A′ on their cells, two without; eight with and one without B′; four with and five without C′; and seven with and two without E′. There was no detectable difference in the strength of the agglutinations of the cells of the backcross offspring from those of the F_1 parent.

Of special interest is the complete correspondence in these nine birds of the presence of C′ and the hybrid-substance (Nos. 2, 6, 8 and 9 with both reactivities, and Nos. 1, 3, 4, 5 and 7 with neither). The probability that C′ and the hybrid-substance segregate independently is $(\frac{1}{2})^9$, or 1:512, if it is assumed that the genetic material responsible for the hybrid-substance does not fractionate. Or, if the 2 × 2 tabular method (FISHER 1944) is used, the probability is 1:126. Hence, it may be concluded that this hybrid-substance is associated with C′ of *livia*.

When the various reagents for the hybrid-substance (from antisera 493F4, 495F6, 480F4, and 491F2) were absorbed with the cells of backcross birds Nos. 2, 6, and 8, all reactivity of the test fluids for the cells of the F_1 (the actual male parent) was removed, indicating that each backcross offspring which carried the hybrid-substance possessed it *in toto*. Further evidence is that the cells of the four backcross hybrids with the hybrid-substance were as reactive as were those of the F_1-*livia-risoria* hybrids when tested with the various fractionated reagents for the hybrid-substance, as given in table 2.

Absorptions of the respective reagents for the species-specific characters of *livia* (A′, B′, C′ and E′) by cells of the backcross birds failed to indicate fractionation of these species-specific factors, implying simple genetic control of these characters as was reported in hybrids of *livia* with *guinea* (MILLER and BRYAN 1953).

Presence of specificities of the hybrid-substance in other species

The reagents prepared from the four antisera to the cells of F_1-*livia/risoria* hybrids possessed antibodies to the hybrid-substance, varying in titer of agglutination from three to eight doubling dilutions with the homologous cells. The cells of eight of thirty species of Columbidae and three other kinds of species hybrids were agglutinated strongly by the reagent for this hybrid-substance prepared from antiserum 493F4, but not at all with the reagents from the other three antisera.

The reagent for the hybrid-substance prepared from antiserum No. 493F4 reacted strongly (3 to 6 doubling dilutions) with the cells of *fasciata*, *rufina*, *luzonica*, *chalcoptera*, *elegans*, *humeralis*, *lophotes*, and *spilorrhoa* and very weakly with some individuals of *senegalensis* (table 2). It also reacted with the cells of F_1-*chalcoptera/elegans*, F_1-*chinensis/risoria*, F_1-*chinensis/orientalis*, and with those of backcross birds containing the d-11 substance from the cross of *chinensis* × *risoria*.

While the cells of certain species and species hybrids were non-reactive with this

TABLE 2

Species and species hybrids possessing the hybrid-substance of the F_1-livia/risoria; *and serological fractionation of some of the specificities of the hybrid-substance from antiserum 493F4*

Test cells	Absorbed with pooled cells of *livia* and *risoria*							
			and 493F4 further absorbed with the cells of:					
	Antisera 3F3 or 182S6	493F4	*fasciata*	*rufina*	F_1-*chinensis risoria*	*lophotes*	*humeralis chalcoptera*, or *elegans*	F_1-*chalcoptera elegans*
livia	0	0	0	0	0	0	0	0
risoria	0	0	0	0	0	0	0	0
fasciata	++	+	0	0	?	0	0	0
rufina	++	++	+	0	+	0	0	0
luzonica		++			+	0		0
humeralis		++	++	+	++	0	0	0
lophotes	++	++	++	+	+	0	0	0
chalcoptera	++	++	++	+	++	0	0	0
elegans	++	++	++	+	+	0	0	0
spilorrhoa		++			+			0
bitorquata	++	±						
capicola	++	±, +						
orientalis	?	±						
semitorquata	+	0						
senegalensis	++	+						
tranquebarica	0	±						
cassini	++							
palumbus	+	?						
asiatica	+	0						
F_1-*orientalis/risoria*	++	±						
F_1-*senegalensis/capicola*	++							
F_1-*bitorquata/risoria*	+							
F_1-*semitorquata/risoria*	+	0						
F_1-*branquebarica/risoria*	0	?						
F_1-*guinea/livia*	+							
F_1-*oenas/livia*	?							
F_1-*chalcoptera/elegans*		++						
F_1-*chinensis/risoria*	++	++	+	0	0	0	0	0
F_1-*chinensis/orientalis*		++		0	0	0		
F_1-*livia/risoria*	+++	+++	++	++	++	+	+	+
s-8, or-8, or hu-8		±						
Column 1	2	3	4	5	6	7	8	9

Additional non-reactive cells are: *chinensis*, *flavirostris*, *guinea*, *maculosa*, *picazuro*, *indica*, *iamaicensis*, *galapagoensis*, *aurita*, *auriculata*, *graysoni*, *macroura*, *nicobarica*, *cristata*, F_1-*fasciata/livia* F_1-*asiatica/risoria*, F_1-*macroura/risoria*, d-4, or-4, and hu-4.

reagent at the usual test dilution (1:60), they were moderately reactive at a lower test dilution of 1:15 (table 2). This was observed for the cells of *bitorquata*, *capicola*, *orientalis*, F_1-*orientalis/risoria*, and F_1-*senegalensis-risoria* with reagent prepared from antiserum 493F4; for the cells of *guinea*, *palumbus*, and of backcross hybrids

(*C/C* or *C/C'* from the cross of *guinea* and *livia*) with reagent from antiserum 491F2, and for the cells of heterozygotes, *C/C'*, with reagent from antiserum 495F6 and 480F4. At a dilution of 1:7.5 the reagent for the hybrid substance prepared from antiserum 493F4 agglutinated the cells of backcross birds carrying s-8, or-8 or hu-8 respectively, as heterozygotes, but not those with d-4, or-4, or hu-4.

Previously unpublished results obtained by PROFESSOR M. R. IRWIN show that two additional antisera to cells of the F1-*livia/risoria* (3F3 and 182S6) contained similar but not identical reactivities to those of antiserum 493F4, (table 2, compare columns 2 and 3). For example, in addition to the reactions mentioned above, his data indicated that the cells of *semitorquata*, *asiatica*, *cassini*, F1-*semitorquata/risoria*, F1-*bitorquata/risoria*, F1-*senegal/capicola*, and F1-*guinea/livia* may contain specificities similar to this hybrid-substance of F1-*livia/risoria*.

Serological fractionation of specificities of the hybrid-substance

Recognition of separable specificities in the reagents for hybrid-substance was possible by absorbing the reagents separately with the reactive cells of certain species and species hybrids (table 2 and 3). As is shown in table 3, cells from both homozygous (*C/C*) and heterozygous (*C/C'*), backcross hybrids of *guinea* × *livia* reacted with reagent for the hybrid-substance from antiserum 491F2 (at a dilution of 1/15 or 1/30); whereas, cells with the other species-specific antigens of *guinea*, (A, B, and E) were not agglutinated, irrespective of whether the cells came from birds homozygous or heterozygous for the respective causative genes. Moreover, further absorption by cells of either homozygous (*C/C*) or heterozygous (*C/C'*) birds removed almost all antibodies for the homologous cells (F1-*livia/risoria*) from this antiserum (i.e., the absorbed fluid was very weakly reactive with the cells of the F1-*livia/risoria*). Therefore, the specificity for the hybrid-substance in this anti-serum is primarily that held in common or "cross-reactive" with the C of *guinea*, and this specificity may be called "a".

The reagent for the hybrid-substance from antiserum 480F4 reacted weakly with

TABLE 3

Further characterization of reagents for the hybrid-substance of F1-livia/risoria

Test cells	Antiserum 493F4				Antiserum 495F6 further absorbed by			Antiserum 480F4					Antiserum 491F2			
	—	CC	CC'	*fasciata*	—	CC	CC'	—	CC	CC'	d-11 or F1 *chinensis/risoria*	d-11 + CC'	—	CC	CC'	*guinea*
F1-*livia/risoria*	+++	+	+	++	++	+	0?	++	+	+	+	+	++	±	±	±
livia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
risoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
guinea	0				0			0					+		0	0
CC	"0"*	0	0		"0"	0	0	"0"	0	0			+	0	0	0
CC'	"0"	0	0	0	+	+	0	+	0	0	+	0	+	0	0	0
d-11 or F1-*chinensis/risoria*	+	+		±	0			+		+	0	0	0			0

Note: cells containing A, B and E characters specific to *guinea* in contrast to *livia*, whether from homozygotes or heterozygotes, do not react with these reagents.

* "0", a reactivity present but demonstrable by absorption only.

cells from the heterozygotes (C/C'), not with those of the homozygotes (C/C); but the absorption with cells from C/C' or C/C birds reduced slightly the reactivity with the cells of F_1-*livia/risoria*, as is given in table 3. This reactivity may be the specificity "a" of antiserum 491F2. The parallel reagent from antiserum 495F6 reacted with cells from heterozygous (C/C') individuals, but not with those homozygous (C/C). Absorptions with cells containing C and C′ almost depleted this antiserum of antibodies for the homologous cells (F_1) leaving only a faint reaction visible microscopically at the 1:15 dilution. The same reagent from antiserum 495F6 further absorbed by cells from homozygotes, C/C (non-reactive at 1:15), however, materially reduced the reaction with the homologous cells (F_1-*livia/risoria*), but left a reactivity with heterozygous cells, C/C'. An additional specificity in this reagent for the cells from heterozygotes (C/C') may be assumed, and this is called "b".

The titer for the cells of the F_1-*livia/risoria* of the reagent from antiserum No. 493F4 was reduced by six doubling dilutions (from 1:7680 to 1:120) by further absorptions with cells from birds homozygous or heterozygous C/C or C/C'. This was quite unexpected, since this reagent failed to agglutinate such cells even at a 1:15 dilution.

To emphasize the more pertinent data presented above; the cells of homozygotes (C/C) or heterozygotes (C/C') produced parallel or nearly parallel results in absorptions of reagents prepared from antisera Nos. 480F4, 491F2, and 493F4, in that the titer was definitely lowered for the homologous cells, F_1-*livia/risoria*. However, these cells from C/C and C/C' individuals differed in their ability to affect the population of antibodies in antiserum 495F6, since the cells of homozygotes (C/C) in absorption removed antibodies for themselves but not for cells from C/C' birds or for those of the F_1-*livia/risoria*. Further, C/C' cells in a similar absorption removed nearly all the antibodies, since only a very faint microscopic reactivity was noted in tests with the homologous F_1 cells. Presumably this reflects corresponding differences in the specificities ("a" and "b") of the antisera.

When the cells of F_1-*chinensis/risoria* or backcross hybrids with d-11 of *chinensis* were used to absorb the reagent for the hybrid-substance prepared from antiserum No. 480F4 (table 3), the reagent still contained antibodies for heterozygous C/C' cells; and when cells from the heterozygotes (C/C') were used in absorption, reactions still occurred with the cells of F_1-*chinensis/risoria* and those carrying d-11. Thus, serum No. 480F4 contains another specificity, "c", of the hybrid-substance of F_1-*livia/risoria*. When the cells of (heterozygous) d-11 and C/C' birds were used together to absorb the reagent from antiserum No. 480F4, the homologous cells, F_1-*livia/risoria*, still were agglutinated by the reagent, indicating a specificity possessed only by them (see table 3). This specificity is labelled "h" and similar antibodies may well be present in the other antisera.

As is given in table 2, column 6, the cells of F_1-*chinensis/risoria* removed reactions from antiserum No. 493F4 for themselves, and for those of F_1-*chinensis-orientalis* (specificity "d"). The titer was reduced by four doubling dilutions (from 1:1920 to 1:120) for the cells of *luzonica* and *lophotes*. A separate specificity responsible for this reduction of titer is not proposed. Little change in titer was noticed for the cells of

spilorrhoa and *fasciata*, and practically no change for the cells of *rufina*, *humeralis*, *chalcoptera*, *elegans*, F_1-*chalcoptera-elegans* or the homologous cells, F_1-*livia/risoria*. (Specificity "d" is different from "c" because reagent from antiserum 480F4 failed to react with *rufina*, *lophotes*, etc., whereas these cells possess "d".)

When the cells of *fasciata* were used to absorb the reagent from antiserum No. 493F4 (table 2, column 4) antibodies were removed only for the cells of *fasciata* (specificity "e"). The cells of *rufina* in a similar absorption removed the antibodies for the cells of *fasciata* ("e"), F_1-*chinensis/risoria* ("d"), and F_1-*chinensis/orientalis* ("d"), but not for the cells of the other species.

The cells of *lophotes*, *humeralis*, *chalcoptera*, *elegans*, or F_1-*chalcoptera/elegans* removed antibodies from the reagent from antiserum No. 493F4 not only for the absorbing cells and for each other but also for all previously reactive cells except those of the F_1-*livia/risoria* (table 2, columns 7 and 8). The cells of these four species then must contain the previous specificities d and e, and at least one other specificity ("f") to account for the reactions in table 2.

The extremely weak reactivity (mentioned earlier) of the cells of *bitorquata*, *capicola*, *orientalis*, and s-8, or-8, and hu-8 from backcross hybrids is indicative of still another specificity, "g", in antiserum No. 493F4. This was confirmed by absorptions with the cells of *orientalis* and *capicola*, each of which removed antibodies for its own cells and the weakly reactive cells of the other species, and for those of the backcross hybrids with s-8, or-8, and hu-8 while antibodies still remained for *fasciata* and *rufina* and the other species with strongly reactive cells. Individual differences were noted within *capicola*, however, for absorption with the cells of one bird did not remove reactivity for those of another. Because of the very weak reactions, the author hesitates to add still another specificity.

The minimum number of specificities of antibodies for the hybrid-substance of *livia-risoria* hybrid contained in antiserum No. 493F4 are "a", "d", "e", "f", "g", and "h"; in antiserum No. 495F6 ("a") and "b"; in antiserum No. 480F4 ("a"), "c", and "h"; in antiserum No. 491F2 "a" and "h". Specificity "a" was demonstrable by absorption only, except in antiserum No. 491F2.

A similar summary of detected specificities possessed by the erythrocytes of the different species is presented in table 4.

Since C of *guinea* was involved in the hybrid-substance specificity, it might be expected that some anti-C reagents would react with the cells of F_1-*livia-risoria* by antigenic specificities shared or "cross-reactive" with those for the hybrid-substance. After absorption with the cells of *livia* and *risoria*, five of twelve antisera to cells from homozygous (C/C) birds and two of three antisera to cells from heterozygotes (C/C') were observed to agglutinate the cells of the F_1-*livia/risoria*, as expected.

Also, some antisera to cells from species which possess one or more specificities of the hybrid-substance might be expected to react with cells of the F_1-*livia/risoria* after absorption with the pooled cells of *livia* and *risoria*. However, an antiserum to the cells of *fasciata*, one against those of *chalcoptera*, two against those of *elegans*, and six against those of *lophotes* did not possess detectable antibodies to the hybrid-substance of F_1-*livia/risoria*. Additional antisera against the cells of these species

TABLE 4

Minimum specificities possessed by the cells reactive with the reagents for the hybrid-substance of F_1-livia/risoria

Test cell	Specificities							
CC	a	·	·	·	·	·	·	·
CC′	a	b	·	·	·	·	·	·
guinea	a	·	·	·	·	·	·	·
palumbus	a?	·	·	·	·	·	·	·
fasciata	·	·	·	·	e	·	·	·
rufina	·	·	·	d	e	·	·	·
humeralis	·	·	·	d	e	f	g	·
lophotes	·	·	·	d	e	f	g	·
chalcoptera	·	·	·	d	e	f	g	·
elegans	·	·	·	d	e	f	g	·
F_1-*chalcoptera/elegans*	·	·	·	d	(e)	f	g	·*
luzonica	·	·	·	(d)	(e)	(f)	(g)	·
spilorrhoa	·	·	·	(d)	(e)	(f)	(g)	·
d-11	·	·	c	d	·	·	·	·
F_1-*chinensis/risoria*	·	·	c	d	·	·	·	·
bitorquata	·	·	·	·	·	·	g	·
capicola	·	·	·	·	·	·	g	·
orientalis	·	·	·	·	·	·	g	·
senegalensis	·	·	·	·	·	·	g	·
tranquebarica	·	·	·	·	·	·	g	·
s-8, or-8, hu-8	·	·	·	·	·	·	g	·
F_1-*livia/risoria*	a	b	c	d	e	f	g	h homologue

* The separate specificities in parentheses were not tested directly and are to be considered merely as possibly present.

might possess such expected cross-reactions. Perhaps the pertinent specificities are not antigenic on cells of these species. Further tests are desirable concerning this expectation.

ADDITIONAL DATA AND DISCUSSION

The parents of several F_1-*livia/risoria* hybrids, three other pigeons and three ring neck doves were given multiple subcutaneous and intra-muscular injections of the cells of the F_1 hybrids. All eight individuals immunized responded with antibodies from very weak (1:1) to strong (1:128) titers. After these antisera were absorbed with the pooled cells of *livia* and *risoria*, antibodies to the hybrid-substance, or the hybrid specificity, were demonstrable in the antisera produced in one ring neck dove and three pigeons (one being a parent of the species hybrids). The strongest reagent for the hybrid-substance from these injections came from the *livia* parent (titer 1:32). Unfortunately there was not sufficient antisera from these birds to conduct additional tests. This demonstration of the ability of the parental species to produce antibodies to the hybrid-substance emphasizes the reality of the specificities present only in the hybrids, probably by virtue of their being heterozygous for genes at one locus.

Recent limited attempts to obtain fertile eggs from matings of *livia* and *risoria* to test for the hybrid-substance in the developing embryo have resulted in only one

hybrid embryo which was bled at approximately 96 hours of age. The cells of this embryo clearly possessed the hybrid-substance, apparently in the same strength as the cells of adult F_1 birds.

BRYAN and MILLER (1953) found that cells from heterozygotes (C/C'), of backcross hybrids of *guinea* to *livia* possessed a hybrid-substance. BRYAN (1953) has observed that *risoria* possessed a C-like substance. Since *livia* contributed the gene or genes for C′ in the cross and *risoria* contributed the gene or genes for a C-like substance, the heterozygous (C'/C-like) F_1-*livia*/*risoria* might be expected to possess a related hybrid-substance. However, BRYAN did not find "cross-reaction" of antibodies for the C/C' hybrid-substance with the cells of the F_1-*livia*/*risoria*. Nevertheless, evidence has been presented in this paper that C and C′ are involved in the specificities of the F_1-*livia*/*risoria* hybrid-substance. This situation may be one of nonreciprocal "cross-reaction" of which one explanation is described by WIENER and WEXLER (1952). The present author has confirmed reciprocal "cross-reaction" of the respective reagents for the hybrid-substance of F_1-*livia*/*risoria* and of F_1-*chinensis*/*risoria*, which was previously reported by IRWIN and COLE (1936b) and IRWIN and CUMLEY (1945).

IRWIN and CUMLEY (1945) found three fractions of the hybrid-substance of F_1-*chinensis*/*risoria*. The first fraction, called dx-A was always associated with the d-11 factor of *chinensis*; dx-B, the second fraction, at best was associated loosely with factors d-1, d-2, d-3, d-7, d-9, d-10 and d-12; and the third fraction, dx-C was always associated with the d-4 factor. The dx-A fraction was observed to be responsible for a large portion of the cross-reaction of the cells of F_1-*livia*/*risoria* (IRWIN 1952). BRYAN (1953) found constant association of the s-8 of *senegalensis*, hu-8 of *tranquebarica* and or-8 of *orientalis* with a hybrid-substance. He also presented evidence that the presence of an allele (s-8′) from *risoria* is always associated with this hybrid-substance. BRYAN reported a series of C-like specificities presumably resulting from a series of C-like alleles in various species including those producing d-11, s-8, hu-8, and or-8. He noted a "cross-reaction" of d-4 cells with reagents for C of *guinea*. Therefore, d-4, and d-11 which apparently are genetically and serologically independent in *chinensis-risoria* hybrids, nevertheless, might be regarded as related. This is not surprising since both d-4 and d-11 have hybrid-substance specificities (dx-C and dx-A, respectively). The cells of backcross hybrids containing or-4, hu-4, s-4 and d-4 (all of which have a hybrid-substance) have not been serologically distinguishable one from another (PALM 1954); and therefore, they are related to C. This leaves only the dx-B fraction "independent" of the C-like specificities. Unfortunately, reagents for dx-B have not been duplicated.

The constant association of the hybrid-substance with species-specific antigens, with the possible exception of dx-B, may be a result of linkage of the causative genes or it may result from "pleiotropic" action of the genes producing the species-specific antigens. The latter hypothesis is favored by the fact that of hundreds of birds tested, no separation of the hybrid-substance from the factors specific to the species has been observed.

Several ideas may be advanced to account for the mechanism of the production of the hybrid-substance.

(1) One may assume that the hybrid-substance is present in one or both parental species "hidden" or "masked" by a position below the surface of the red blood cells but is on or near the surface of the cells of the F_1. Presumably rabbits would engender antibodies to subsurface antigens of the cells as well as to those of the surface. Therefore, one test of this possibility is to absorb antisera to cells of each parental species with the cells of both parental species and test the resulting reagent with the cells of the F_1-hybrid. Ten anti-*livia* and six anti-*risoria* sera have been so absorbed and the resulting fluids failed to agglutinate cells of the F_1-*livia/risoria* at a dilution of 1:15 or through four doubling dilutions beyond. Similar tests by PALM (1954) with antisera to *chinensis* have failed to show antibodies for the hybrid-substance of the F_1-*chinensis/risoria*.

A similar test of this hypothesis was conducted by pooling cells of *livia* and *risoria* before injection. Six rabbits received such pooled samples of whole cells and six others received similar samples which before injection had been lysed by freezing or by adding distilled water and returning to salinity (about 0.9%). After absorption of the twelve antisera so produced with pooled whole cells of *livia* and *risoria* no reactivity remained for the cells of F_1-*livia/risoria*.

The assumption presented above of a "sub-surface" antigen in the cells of one or both parental species is not disproved by these two experiments, since lysis of the cell *in vitro* or *in vivo* may not properly uncover a "hidden" antigen. Nevertheless, negative evidence certainly does not argue *for* the "sub-surface" assumption.

Treatment of the parental cells with trypsin (or other enzymes) might alter them so as to reveal specificities of the hybrid-substance. Following the method of ROSENFIELD and VOGEL (1951), trypsin and papain treatment of the cells of several individuals of *livia*, *risoria*, and the F_1-hybrids was conducted. The cells of *livia* and *risoria* following such treatment were not reactive, nor could a change other than a very slight enhancement of the agglutination of the cells of the F_1 be detected in the agglutination tests of these kinds of cells with the four reagents for the hybrid-substance.

The ability of the parental species to produce antibodies to the hybrid-substance also argues against their possessing it below the surface of the cell. While animals may produce antibodies to their own tissues, such tissues are relatively isolated from the blood circulation, whereas erythrocytes are continually being broken down and in contact with the reticulo-endothelial system.

(2) One might assume that a recessive inhibitor in one parental species suppresses the specificities of the hybrid-substance on the cells. Or, possibly, a dominant inhibitor from one species prevents final or full expression of an antigen normal to the other species and the hybrid-substance is merely an intermediate or alternate product.

We now know that phenomena exist which are consistent with the idea that inhibitor genes may affect antigenic factors. RENDEL, *et al.* (1953) present evidence for a recessive inhibitor which is epistatic to the gene producing R (and probably to that producing r) of sheep. LEVINE, *et al.* present a similar case for the A B O system of humans. FOX and WHITE (1953) present data for a disappearance of antigen 1 (a hybrid-substance-like specificity in Drosophila) which is consistent with the assumption that a mutant inhibitor occurred. The inhibitor may be regarded as a mutant of

a relatively independent gene-enzyme system which now can "block" the action of the gene producing the antigen. Or, as FOX and WHITE indicate, the inhibitor may be regarded as a mutant of a gene actually involved in the synthesis of the antigens in question. It would be expected, however, contrary to most of the observed data, that a hybrid-substance in segregation would *not* give a 1:1 ratio in the backcrosses (e.g. d-11 IRWIN and CUMLEY 1945), unless the postulated inhibitor were closely linked to the gene producing the species-specific antigen.

(3) One might assume that nothing new is produced except a different physical position, (a new propinquity) of the antigens on the erythrocytes of the hybrid, which in the animal immunized stimulates or permits the antibodies to be formed against the combination, resulting in a new specificity of some of the antibodies. This has been suggested previously by BURNET and FENNER (1948). If so, one would expect that the hybrid-substance would be present only when genes (alleles) producing different (species-specific) antigens were present. The dx-B fraction of the hybrid-substance of F_1-*chinensis/risoria* (IRWIN and CUMLEY 1945) exhibited, at best, a loose association with *known* species-specific antigens or factors of *chinensis* in contrast to *risoria*; and thus provided some evidence against the current assumption. However, not all the species-specific factors of *chinensis* necessarily were recognized and dx-B could be correlated with an unrecognized antigen.

From the same assumption one might expect that almost any heterozygote for genes producing antigens would induce "hybrid-substance" specificities (BRYAN and MILLER 1953). Apparently this is not true for heterozygotes, E/E' and B/B' of the cross of *guinea* with *livia* (MILLER 1953), but such tests with other heterozygotes for blood factors are few or lacking.

(4) The possibility exists of interaction between complementary genes of closely linked loci. The addition of another biochemical chain or step may lead to a new or different end product. Of course, the latter is not limited to the idea of complementary genes. However, again it is not necessary to postulate a new product. A resemblance between the idea of interaction of complementary genes and the above hypothesis (3) may be noted if one recalls the classic factor interaction of the "rose" and "pea" comb in chickens. The resulting F_1-"walnut" comb certainly has a different configuration than either "parent" type.

(5) One important hypothesis remains. An interaction of alleles or allelic gene products might produce an entirely new substance. No experimental test of this hypothesis yet has occurred to the author. Chemical isolation of the hybrid-substance would aid in excluding some of these hypotheses. Indeed, as implied above (4 and 3), no direct evidence exists that the hybrid-substance is really a substance, per se.

It has been shown that C of *guinea* was involved in specificities of the F_1-*livia/risoria* hybrid-substance, also, that C′ appears to be necessary for the presence of the hybrid-substance. BRYAN (1953) has reported that *risoria* has a C-like antigen. Since this C-like antigen of *risoria* was the "constant" in the unique backcross family of *livia/risoria*-hybrids, it was to be expected, if the hybrid-substance resulted from some sort of interaction of genes producing species-specific antigens, that the segregation of the hybrid-substance would follow an antigen of *livia* (C′).

If C-like and C′ alleles, or genes closely linked to them, are truly the genetic basis

for the hybrid-substance; then offspring of matings of homozygous *CC* hybrids (derived from *guinea*) mated with *risoria* should lack part of the hybrid-substance of the erythrocytes of F_1-*livia/risoria*. Such hybrids might also possess new hybrid-substance specificities. Matings to produce these hybrids have not yet produced viable offspring.

The present paper has presented immunogenetic evidence that with but one possible exception, the specificities of the hybrid-substance in Columbiformes are associated with the C series of specificities. (If this were extended to all hybrid-substances, it would require that ducks have a C-like locus, since their species-hybrids may have a hybrid-substance; see McGibbon 1944, 1945. Also, Sokolowskaia (1936) may have detected a hybrid-substance in the serum of species hybrids of ducks.) It is possible that the association is "spurious." If d-4, d-11 and the C of *guinea* each are composed of 2 or more specificities controlled by closely "fortuitously" linked genes, further, if d-4 is made of specificities we may call "a" and "b" and d-11 is composed of "c" and "d" and C is composed of "b" and "c", then a "cross-reaction" of reagents for C with d-4 and d-11 need not mean genetic or serological relationship and the "association" would be "spurious." However, in the author's opinion this explanation is unlikely.

It has been noted that the cells of certain species possessed one or more specificities related to the hybrid-substance of certain other species crosses. For example, *lophotes* possesses certain specificities of the hybrid-substance of the cells of F_1-*livia/risoria*. Several proposals have been made by Bryan (1953) to account for the possession of the hybrid-substance as a normal feature of cells of certain species: "(a) The species-specific substances possess some molecular configurations which make them cross-reactive with the hybrid-substance; (b) a single gene in certain species produces the same substance which is produced as an interaction product by two genes from two separate species; (c) the two genes giving rise to the hybrid-substance may exist in certain species at two different loci on two different chromosomes; that is they are nonallelic; (d) the genes interacting to produce the hybrid substance are closely linked and occur in certain species in a homozygous state; (e) some species maintain a state of perpetual heterozygosity for the genes which interact to produce the hybrid substance, by possessing a system of balanced lethals."

The last proposal was recognized as very unlikely. At present, pertinent information is too meagre to judge among these and other possible explanations.

SUMMARY

The blood cells of hybrids between the domestic pigeon, *Columba livia*, and the ring neck dove, *Streptopelia risoria*, possess a "hybrid-substance". That is, they possess a serological reactivity not present in cells of either parent.

Immunogenetic relationships of the "hybrid-substance" of the F_1-*livia/risoria* were demonstrated with other species and hybrids of Columbiformes. The erythrocytes of 17 of 34 species reacted with reagents for this "hybrid-reactivity" prepared from antisera against the cells of the F_1-*livia/risoria*. Ten of 15 kinds of F_1-hybrids of other species crosses also were shown to possess some antigenic specificities related to those of the hybrid-reactivities of the F_1-*livia/risoria*. By appropriate absorptions

and subsequent agglutination tests the reagents for the "hybrid-substance" were fractionated into a minimum of eight specificities, one of which was related to antigen C of *guinea*, and another with heterozygotes, *C/C'*.

Segregation of species-specific antigens of the domestic pigeon, *Columba livia*, and of the "hybrid-substance" in a unique family from a mating of F_1-*livia/risoria* with the ring neck dove, *Streptopelia risoria*, indicated that the C' character of *livia* was always associated with the hybrid reactivity of this generic cross. Presumably, the "hybrid-substance" results from the presence of a gene for C' together with a gene for a C-like antigen (of *guinea*) in *risoria*, or from genes linked to these.

The specificities of the hybrid-substance were not detectable in cells of the parental species even when the cells were "altered" by enzyme treatment; nor was there evidence that the hybrid specificities were present in the parental species, but located beneath the surface of the cell, rather than at the surface.

Some possible mechanisms for the production of the "hybrid-substance" were discussed: (1) Subsurface position in the cells of the parents; (2) An inhibitor present in one species; (3) New arrangement of antigens in the hybrid resulting in new specificities; (4) Interaction of closely linked complementary genes; and (5) Interaction of alleles producing a new substance.

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BEHAVIOR OF NUCLEI AT CONJUGATION IN PARAMECIUM AURELIA. II. THE EFFECTS OF X-RAYS ON DIPLOID AND HAPLOID CLONES, WITH A DISCUSSION OF DOMINANT LETHALS[1]

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Received March 30, 1956

AT conjugation in *Paramecium aurelia*, the two diploid micronuclei in each member of the conjugant pair undergo meiosis to produce eight products. One of these products enters the cytoplasmic region known as the paroral cone and survives; the rest disintegrate. The nucleus in the cone divides mitotically to form the male and female gamete nuclei. The male gamete nucleus from each conjugant passes into the other and fuses with the female nucleus which has remained behind. For successful completion, this process requires a series of rather precisely oriented nuclear movements and precisely timed divisions.

KIMBALL and GAITHER (1955) have shown that, in comparison with normal nuclei from diploids, deficient nuclei produced by meiosis in haploids are at a selective disadvantage in entering the paroral cone. Deficient nuclei also frequently fail to form functional male gamete nuclei although they usually form female ones. The rather similar effects on nuclear behavior caused by X-rays will be discussed in this paper. However, it will be shown that only part of these effects can be caused by X-ray-induced deficiencies. The occurrence of dominant lethals in Paramecium is also discussed.

METHODS

Stocks and crosses

The stocks and general crossing and testing procedures have been described in the first paper of this series. The procedure in the present work has been to irradiate animals of stock 90, or its derivatives, and mate them with stock CD animals, either immediately after irradiation or one or two days and several fissions later. Three kinds of clones of stock 90 origin were available and have been described in the first paper—clones with two diploid micronuclei, a clone with two haploid micronuclei (clone 3b), and a clone with one diploid and two haploid micronuclei (clone 20b). All these clones had the wild type alleles of the genes clear (*cl*), dumpy (*dp*), and mating type (*mt*) and the 90 alleles of the antigen genes *d* and *g*. Stock CD had the recessive alleles of the first three, and the 60 alleles of *d* and *g*. It regularly had two diploid micronuclei. In one experiment, amicronucleate animals of CD origin were used.

Each exconjugant was isolated separately, and the cytoplasmic origin ascertained by use of *cl* and *dp*, as previously described. Four days later, the culture from each isolated animal was classified for amount of growth and for the presence or absence of crystals (lacking in animals without cl^+). In addition, tests for either the D or the

[1] Work performed under Contract W-7405-eng-26 for the U. S. Atomic Energy Commission.

G antigens were often made. Heterozygotes react with both anti-60 and anti-90 serum; homozygotes react with one or the other only.

Irradiation procedures

Small groups of animals of the desired stock were irradiated in small hanging drops in a chamber containing pure nitrogen or 20 percent oxygen and 80 percent nitrogen. Larger groups were irradiated in small lucite containers with air above the shallow layer of fluid.

A G-E Maxitron 250 X-ray machine was used. Exposures were made at about 1500 r/min at 250 kvp and 30 ma with 3 mm of Al added filtration (hvl, 0.55 mm Cu) or at about 16,000 r/min at 250 kvp and 30 ma but with no added filtration (hvl was small but not precisely known; dose measurements were made with a nylon Victoreen thimble chamber). Unless otherwise stated, the results are for the first procedure.

RESULTS

Stock 90 diploids irradiated with 33 kr

Table 1 shows the combined data for a series of experiments in which stock 90 diploids were irradiated with about 33 kr and then, either immediately or two days later, crossed with unirradiated CD. The pairs are classified according to cytoplasmic

TABLE 1

Classification of pairs from matings of irradiated (about 33 kr) or unirradiated stock 90 with stock CD

Line	Inferred origin of nucleus of clone from†		Classification for growth*									
			Number of pairs in each category									
			Irradiated					Control				
	CD conjugant	90 conjugant	G/G	G/P	P/G	P/P	Total	G/G	G/P	P/G	P/P	Total
1	CD & 90	CD & 90	474	37	12	26	549	332	3	0	0	335
2	CD & 90	CD	1	0	0	0	1	2	0	0	0	2
3	CD & 90	90	34	7	2	3	46	6	1	0	0	7
4	CD & 90	?	1	20	1	1	23	0	3	0	0	3
5	CD	CD & 90	240	81	45	14	380	5	1	1	0	7
6	CD	CD	99	8	3	6	116	0	1	0	0	1
7	CD	90	55	76	12	18	161	8	0	3	0	11
8	CD	?	9	151	1	33	194	0	2	0	0	2
9	90	CD & 90	0	0	2	0	2	2	0	0	0	2
10	90	Other	0	0	0	0	0	0	0	0	0	0
11	?	CD & 90	0	0	18	4	22	0	0	1	0	1
12	?	CD	0	0	2	0	2	0	0	0	0	0
13	?	90	0	0	9	5	14	0	0	0	0	0
14	?	?	0	0	0	20	20	0	0	0	0	0
Totals.....			913	380	107	130	1530	355	11	5	0	371

* G means that the clone attained a maximum population in four days, P that it failed to attain a maximum population in this time. The symbol to the left applies to the clone from the CD conjugant; the symbol to the right, to that from the 90 conjugant.

† See text.

and nuclear origin and the amount of growth in four days. About a third of the clones of CD cytoplasmic origin were tested for serotype. In all except two (line 9 of the table), clones with crystals reacted with both anti-60 and anti-90 serum, which indicates that they obtained nuclear contributions from both parents. The two exceptions reacted with anti-90 serum only, which suggests a contribution from 90 only. Less than one percent of the clones without crystals reacted with both antisera; the rest reacted with anti-60 only. Thus almost all the clones without crystals acted as though they had received a nucleus from CD only. The remaining two thirds of the clones were, therefore, classified as having nuclei from both parents if they had crystals, and from CD only if they did not. This would introduce only a small error over the double classification for crystals and serotype.

Clones of 90 cytoplasmic origin were tested for serotype whenever possible, and the nuclear origin was inferred from the serotype. Clones which reacted with both anti-60 and anti-90 serum were considered to have hybrid nuclei (CD & 90); clones which reacted with only anti-90 serum, nuclei from 90; and clones which reacted with only anti-60 serum, nuclei from CD. Less than one percent of the clones reacted with only anti-60 serum, but had crystals; these were classified as hybrid. There were also a few clones which lacked crystals but reacted with both antisera.

The classification is quite accurate for clones with good growth, but phenotypic lag introduces errors for clones with poor growth. KIMBALL and GAITHER (1955) have shown that only about ten percent of the poorly growing clones from crosses with haploids fail to gain a character for which they have gained a gene, but about 75 percent fail to lose a character for which they have lost a gene. These estimates were based only on clones in which tests for serotype could be made, and the error may have been greater for clones of CD cytoplasmic origin whose nuclear origin was ascertained only from crystals because some of these grew so poorly that a test for serotype would not have been possible. The classification as hybrid when crystals were present was probably correct in almost all cases since failure to obtain a nucleus from unirradiated CD should have been rare. However, an appreciable part of the clones classified as having nuclei from CD only may have been hybrids. It should be emphasized that the poorly growing clones in the present experiment had better growth, on the average, than those from the haploids; therefore, errors due to lag were probably less in those clones that were tested for serotype than in the haploid experiments.

A diagrammatic interpretation of three of the classes of pairs in table 1 is given in figure 1. The pairs in line 1 of the table apparently had normal exchange of gamete nuclei. It is somewhat remarkable that even at the high dose of 33 kr about a fourth of all the pairs were normal in both nuclear behavior and growth (line 1, column G/G). The pairs on line 5 probably arose from a one-way transfer of gamete nuclei as shown in the diagram. The interpretation is supported by one experiment in which the clones from the CD member of all 20 pairs in this category were found to have small micronuclei and to produce inviable progeny at autogamy. These are the characteristics expected of clones with haploid micronuclei derived from a female gamete nucleus of CD origin. Moreover none of the progeny by autogamy developed crystals. This shows that the clones did not have a macronucleus derived by regeneration from

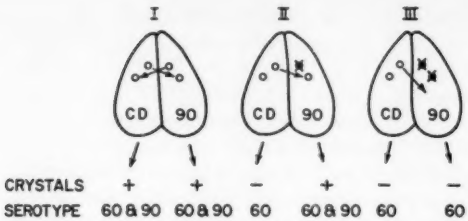

FIGURE 1.—Diagrammatic interpretation of the behavior of gamete nuclei for three classes of pairs in table 1. The phenotype of the exconjugant clones is shown below the diagrams. Class I corresponds to line 1 of table 1; class II, to line 5; and class III, to line 6.

the original CD macronucleus combined with hybrid micronuclei or haploid micronuclei of 90 origin. The pairs on line 6 probably derived their nuclei entirely from CD as a result of failure of the irradiated conjugant to furnish any functional gamete nuclei. This interpretation is confirmed by the observation that the clones with good growth in this category had the phenotype expected if they had obtained only the recessive alleles of *dp* and *mt*. It is worth noting that this category of pairs was not very frequent even at higher doses than 33 kr. Inactivation of all nuclei seems to be difficult or impossible to achieve by X-irradiation.

There are two other major categories of pairs, those on line 7 and those whose nuclear origin could not be completely ascertained. The pairs on line 7 show no evidence of nuclear exchange. This result could be obtained in a number of ways which are not worth detailed discussion. It is enough to point out that the clones of 90 origin with good growth must have derived their macronuclei by regeneration from the old macronuclei or have undergone no reorganization; otherwise they should have grown poorly because of deleterious recessive mutations. Attempts were made to avoid pairs that separated without reorganization, but they were probably not completely successful. Pairs without evidence of nuclear exchange were not much more frequent at 50 and 60 kr than at 33 kr. Most of the clones of undetermined nuclear origin on lines 4, 8, and 11 through 14 died before adequate observations could be made. They undoubtedly arose by a variety of nuclear events and should be considered in estimating the frequency of clones with poor growth in the other categories. However, this would be difficult to do and is unnecessary for present purposes. The greater number of clones of unknown nuclear origin among the descendants of the 90 conjugants is, in part, a consequence of a real difference in viability but is also a consequence of the greater difficulty in establishing the nuclear origin of such clones.

Information from clones of CD cytoplasmic origin

Five types of clones from the CD cytoplasmic parent can be recognized by routine observations without serum tests. These are clones with crystals and normal growth (G+), clones with crystals and poor growth (P+), clones without crystals and normal growth (G−), clones without crystals and poor growth (P−), and clones that died before classification (N). The discussion of the 33-kr group shows that most of the clones with crystals (G+ and P+) were hybrids, and those with good growth

and no crystals (G−) derived their nuclei from a single gamete nucleus from CD. Because of phenotypic lag, the P− clones could have derived their nuclei from both parents, or only from CD. The frequency of these five types of clones can be used to ascertain the frequency of various nuclear contributions from the irradiated mate. The G+ and P+ clones and some of the P− and N ones obtained a functional gamete nucleus from the mate but the G− and some of the P− and N did not.

Oxygen concentration, intensity, time of mating

The data in table 1 came from a series of experiments in which certain features of irradiation, auxiliary treatment, and subsequent handling were varied. Since none of the variations had any significant effect on the outcome, the data were combined. However, the data for the CD member of the pair are summarized separately for the various treatments in table 2. All the data are also included in table 1 except one of the high-intensity experiments in which the 90 member was not followed.

The chi-squares for homogeneity of the first two comparisons in table 2 are not significant at the five percent level. KIMBALL (1955) has given dose curves for the percentage of clones with crystals (G+ and P+) that show that the same lack of an oxygen effect holds at all doses. The intensity comparison was not designed as a critical test since the high intensity irradiation included a larger soft component than the low, and so may not have been measured as accurately. However, the results show that experiments done under the two conditions can be combined and an intensity effect, if it exists, must be small.

In three of the experiments included in table 1, part of the animals were mated immediately after irradiation and part were allowed to multiply for two days (between 4 and 6 fissions) before they were mated. The chi-square for homogeneity is significant at the one percent level, but this is entirely because of the P+ and N classes, as can be seen from table 2. Most of this discrepancy was caused by one experiment of the three, and it may be suggested that, for some reason, the conditions for temporary survival of poorly growing clones were especially bad in the delayed mating group. In any case, most of the effects of the radiation are inherited essen-

TABLE 2

Frequency of clones of CD cytoplasmic origin for various phenotypes, clones from the irradiated group in table 1 grouped according to treatment

Treatment	Percentage of clones in category*					Total clones
	G+	P+	G−	P−	N	
Oxygen present	34.9	2.9	49.8	9.0	3.3	785
Oxygen absent	40.1	3.3	43.9	8.2	4.4	745
X-rays, 1.5 kr/min, 3-mm Al filter	37.2	2.9	47.3	8.7	3.8	1410
X-rays, 16 kr/min, no filter	45.6	3.9	40.0	7.2	3.3	180
Immediate mating	42.1	4.5	43.4	8.8	1.1	442
Delayed mating	38.4	1.5	46.0	7.6	6.5	474

* See text.

tially unchanged over several fissions and, quite possibly, indefinitely. Matings were made immediately after irradiation in all experiments included in table 1 except the three just discussed. However, in some of the experiments on dose relations and in all crosses with 3b and 20b to be described in a later section, matings were made one or two days after irradiation.

Tests for a cytoplasmic effect of radiation

A direct test for a cytoplasmic effect of radiation on postconjugation growth was made by irradiating amicronucleate animals and introducing an unirradiated nucleus into them by conjugation. The amicronucleate line was of CD origin and so lacked crystals. It was also smaller than normal, grew slowly, and often produced inviable sublines. These features have characterized all the amicronucleate clones we have obtained by exposing CD conjugants to 40°C. A part of the amicronucleate line was irradiated with 35 kr, and another part was kept as a control. A day later both groups were mated with unirradiated stock-90 animals. Data are given in table 3 for the exconjugants of amicronucleate cytoplasmic origin. Those exconjugants that developed crystals must have obtained a nucleus from 90, and this conclusion is supported by the fact that they were all of normal size. The exconjugants that did not develop crystals probably obtained no nucleus from 90, and this conclusion is supported by their small size. Most of the exconjugants that died probably also failed to get a nucleus from 90. The large number of such failures may well be related to the disparity in size between the amicronucleate and micronucleate members of the pairs, for this would make proper alignment during conjugation difficult. From the present point of view, the important fact is that there is no indication of an effect of radiation on growth and survival after conjugation.

In principle, a test for a cytoplasmic effect of radiation is also afforded by the pairs on lines 1 and 6 of table 1. The two members of these pairs should have had identical nuclei and so should have differed only in cytoplasmic origin. Apparently, there is a cytoplasmic effect since more clones are found in the G/P than in the P/G category. However, this probably results from some sort of incompatability reaction between the genes of one stock and the cytoplasm from another and has nothing to do with radiation. It has been observed repeatedly in crosses of unirradiated CD and 90 that the F_2 by autogamy contains more clones which die or grow poorly than F_2's within the stocks and that the frequency of such clones is greater in the descendants of the 90 than of the CD members of the pairs. The excess of G/P over P/G clones among the controls in table 1 points in the same direction.

TABLE 3

Frequency of exconjugants of amicronucleate cytoplasmic origin

Treatment	Percentage of clones in category*					Total clones
	G+	P+	G−	P−	N	
Amicronucleate irradiated	5.7	13.3	0.0	26.7	54.3	210
Amicronucleate unirradiated	4.1	9.4	0.0	27.6	58.8	170

* See text.

Dose

The dose relations of the five types of clones of CD cytoplasmic origin are shown in figure 2. Two conclusions are obvious. X-rays inhibit the formation of functional male gamete nuclei as shown by the nearly linear increase of G− clones, and at no dose is there a large number of the clones (P+, N, P−) that could be caused by dominant lethals. Thus dominant lethals are selected against in the formation of male gamete nuclei, produced in relatively small numbers, or, more probably, both.

It has already been shown that radiation-induced deficiencies, as detected by loss of one but not others of the marker genes, are not common at 33 kr. Most of the clones from the 50- and 60-kr groups were tested for serotype as well as for the presence of crystals and amount of growth. A few instances, some with good and some with poor growth, were found in which d^{90} or cl^{+}, but not both, were transmitted from 90. In addition, all G− clones at all doses were observed for shape, and a few were found which were normal instead of dumpy and so must have obtained the dp^{+} but not the

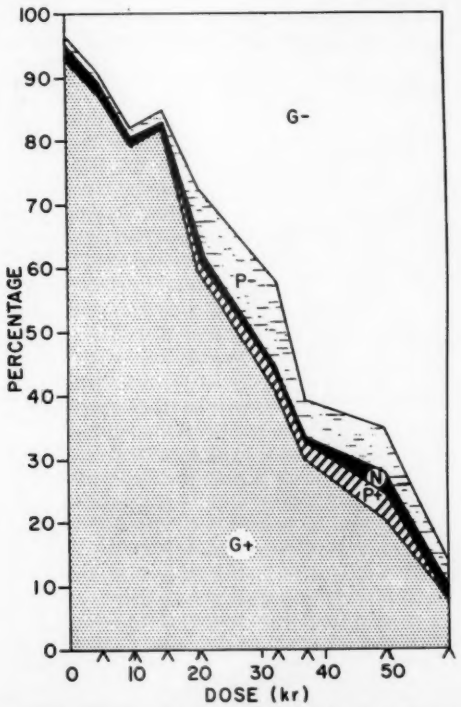

FIGURE 2.—Percentage frequency of phenotypes among clones of CD cytoplasmic origin from crosses of unirradiated CD with irradiated diploid 90. The data are plotted as cumulative percentage frequency starting with G+ and adding in order P+, N, P−, and G−. The doses for which the data were obtained are marked on the abscissa. The meaning of the symbols is given in the text.

cl^+ gene from 90. However, by considering all the data, the frequency of deficiencies for any one locus was less than one percent at any dose. Thus, even at the highest doses, the extent of chromosome loss was small compared to the loss of about half a chromosome set that occurs at meiosis in haploids.

Crosses with clones having haploid micronuclei

Figure 3 shows the results of crossing unirradiated CD with irradiated 3b, a clone of stock 90 origin with two haploid micronuclei. The frequencies of the five classes of clones of CD cytoplasmic origin are shown in a manner similar to figure 2.

As was shown in the first paper, the male gamete nucleus from the unirradiated haploid fails to function in between a third and a fourth of the pairs. The remaining clones are probably hybrids that grew poorly as a result of extensive aneuploidy. About half have crystals and half do not, as expected with random segregation of the single haploid set at meiosis.

The two main features in figure 3 are the rapid increase of G− clones with dose and the relatively small change in P− clones. Comparison with figure 2 shows that the increase of G− clones was more rapid at intermediate doses in the haploid than

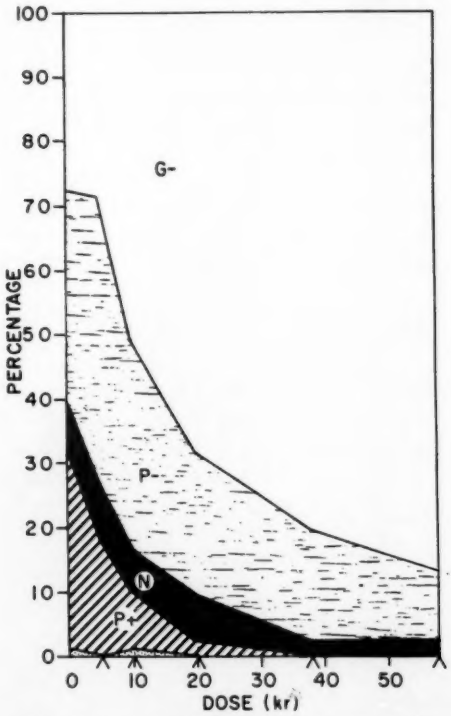

FIGURE 3.—Same as figure 2 except that the irradiated clone (3b) had two haploid micronuclei.

the diploid cross. The more rapid leveling off at high doses in the haploid crosses is not meaningful, since P− clones of CD nuclear origin may have been more frequent because different samples of CD were used. The approximate constancy of the P− clones over the dose range studied is in part explained by an increase in poorly growing clones with nuclei from CD only, concomitant with a decrease in P− clones of hybrid origin. However, especially at intermediate doses, P− clones were too numerous to be entirely accounted for in this way. It seems probable that hybrid clones that fail to develop crystals because of phenotypic lag account for at least part of the discrepancy.

Figure 4 shows the results of mating unirradiated CD with irradiated 20b, a clone of the same origin as 3b except that it contained a diploid in addition to two haploid micronuclei. The irradiations and matings were made at the same time as those with 3b, and the same culture of CD was used for the matings. In the first paper, some of these data were used to show that the unirradiated diploid micronucleus has nearly

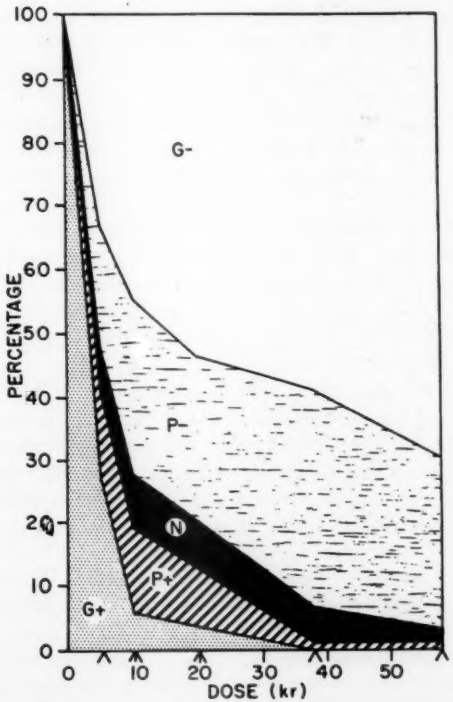

FIGURE 4.—Same as figure 2 except that the irradiated clone (20b) had one diploid and two haploid micronuclei. Through an error in handling, only data for the frequency of G− and non-G− clones were obtained at 20 kr. Therefore the other points at 10 and 38 kr were connected with straight lines.

complete selective advantage over the haploid in forming the gamete nuclei and that the three nuclei appear to contribute gamete nuclei with equal frequency after 5 kr. However, figure 4 shows marked departures from equal contributions after a further increase in dose. In the first place, there are too few G+ clones if the expectation is obtained by combining figures 2 and 3 in a proportion of 1:2. No hypothesis involving some form of selection of the more complete products can be made to fit unless it is assumed that the haploid micronuclei have an advantage in relation to the diploid at higher doses. The alternative remains that the diploid nucleus in clone 20b was, for some reason, more sensitive than those in the several clones on which figure 2 is based. Some variation in sensitivity among diploid clones was found, but it was insufficient to account for the low frequency of G+. Nevertheless, an unusually sensitive diploid micronucleus is not entirely excluded.

The second departure from expectation is the large number of P− clones at high doses. These clones could not have arisen entirely through simple failure of nuclear exchange, since the frequency relative to G− should, then, have been no higher than in figure 3. It seems probable that these P− clones were hybrids that failed to develop crystals because of phenotypic lag, but the reason for the large number is not clear.

DISCUSSION

The poor growth of hybrid clones can be interpreted as a dominant lethal phenomenon resulting from nuclear rather than cytoplasmic damage. Since most of the poorly growing clones eventually die, no distinction is warranted between dominant deleterious and dominant lethal effects. The amicronucleate crosses provide evidence that the phenomenon is mainly nuclear. The results are in general agreement with WHITING's findings (1955) in Habrobracon that nuclear effects are produced at considerably lower doses than cytoplasmic effects. Indeed, the doses required to produce a postfertilization cytoplasmic effect in Paramecium are probably considerably greater than in Habrobracon, but this is not surprising since a dose of about 300 kr is required to kill vegetatively multiplying paramecia.

It seems probable that part, perhaps all, of the poor growth of hybrids is the result of dominant effects of loss of chromosome parts. This is likely on *a priori* grounds since a considerable amount of chromosome loss would be expected at 33 kr. It is supported by the finding that the two members of a pair are alike in growth more often than would be expected by chance when both are hybrid (line 1, table 1). The many discrepant pairs on line 1 are not unexpected, since phenotypic lag in the development of poor growth would probably not be the same in the two members of the pair and the previously mentioned cytoplasmic difference between the two stocks might even result in persistent differences in response to certain chromosome losses. The correlation between poor growth of hybrid clones of 90 cytoplasmic origin and failure of the male gamete nucleus to function (compare lines 1 and 5, table 1) also supports the chromosome loss interpretation. This correlation is to be expected for chromosome losses as shown by KIMBALL and GAITHER (1955).

Estimation of the frequency of dominant lethals is made difficult by (1) the selection against them, which undoubtedly occurs during conjugation; (2) the problem of interpretation of the discrepant pairs which may or may not be instances of dominant

lethals expressed, at least during the first few days after conjugation, in only one member of the pair; and (3) the uncertainty about the number of clones of undetermined nuclear origin that should be included. Despite these difficulties, it is possible to obtain a maximum estimate from table 1 since about half the clones from the 90 member were hybrids with good growth. If it is assumed that only one of the eight meiotic products of the two micronuclei need be free of dominant lethals to be sure of producing a hybrid with good growth, then the maximum frequency of the meiotic products containing dominant lethals is 92 percent. The actual value is probably lower.

Even this maximum value for the frequency of dominant lethals is considerably lower than would have been expected from the data for Drosophila sperm (e.g. LEA 1955). KIMBALL (1949) has shown that recessive lethals are induced in Paramecium with approximately the same frequency as in Drosophila sperm; there is, therefore no real reason for believing that chromosome breakage is low in Paramecium. Elimination of dominant lethals before conjugation could not be involved, since no death of the animals occurs, and many of the matings were made immediately after irradiation without intervening multiplication. Elimination during conjugation undoubtedly occurs but has been taken into account in the calculation of the maximum value.

The explanation probably is that only a small part of the chromosomal aberrations induced in Paramecium act as dominant lethals. Since there are about 40 pairs of chromosomes (DIPPELL 1954), one break could cause an average loss of no more than one fortieth of a set. Furthermore, the organism is fairly resistant to the deleterious effects of aneuploidy. KIMBALL and GAITHER (1955) found, among the progeny of crosses between haploids and diploids, several clones with good growth which were certainly aneuploid for one chromosome, and probably for several. The high degree of polyploidy of the macronucleus may make aneuploidy less detrimental than in many organisms.

As in the previous paper, two types of disturbance in nuclear behavior can be detected, alteration in the selective advantage for entering the paroral cone and failure to form a functional male gamete nucleus. The evidence concerning the former comes from the crosses with clone 20b; the evidence for the latter, from the frequency of G− clones in the progeny of the CD members of the pairs.

The first question is how much of the effect of X-rays on nuclear behavior can be attributed to induced chromosome losses. KIMBALL and GAITHER (1955) conclude that the disturbances in nuclear behavior in unirradiated haploids result from the extensive chromosome losses brought about by random segregation of a single chromosome set at meiosis. In the present work, the association of dominant lethality in the clone from the irradiated member with failure to contribute a male gamete nucleus to the mate favors the loss hypothesis. So does the rapid decline in selective advantage of the diploid micronucleus of 20b in the dose range below 5 kr, since the frequency of meiotic products with recessive lethals increases rapidly in this range (KIMBALL 1949). However the extent of induced deficiencies is much less, even at very high dose, than those produced by meiosis in the unirradiated haploid. For this reason, deficiencies cannot explain the more frequent failure of the male gamete nucleus in heavily irradiated diploids than in unirradiated haploids, nor the more rapid increase

of such failures with dose in the haploid than in the diploid. Neither can they explain the apparent reversal in selective advantage of diploid and haploid micronuclei in heavily irradiated clone 20b. Thus it seems probable that induced deficiencies are responsible for some, but not all, of the effects of radiation on nuclear behavior.

KIMBALL and GAITHER (1955) concluded that anaphase bridges could not be important in disturbing nuclear behavior in haploids. The bases for this conclusion were (1) that it is unlikely that anaphase bridges would slow nuclear separation appreciably since the spindles are very large compared to the chromosomes; and (2) that small delays, which were all that the cytological observations allowed, would not be sufficient to influence appreciably the entrance into the paroral cone. The same arguments can be applied to the X-ray case, since no detectable delay in meiosis was found by cytological examination after 33 kr. Moreover, bridges cannot account for the greater sensitivity of haploids to inhibition of the exchange of male gamete nuclei since more bridges would be expected in diploids than in haploids.

SONNEBORN *et al.* (1953) have attributed similar abnormalities in nuclear behavior induced by ultraviolet to a slower meiosis in the irradiated mate. It appears from their brief account that complete failure of gamete nuclei from the irradiated mate (similar to the pairs on line 6 of table 1) was more common with ultraviolet than with X-rays, and they do not specifically mention one-sided nuclear exchange with a male nucleus reaching the irradiated but not the unirradiated mate. Therefore, it seems probable that the kinds of events, or at least their relative frequency, were different in the two cases. Cytological observations in this laboratory do not suggest that the irradiated animals underwent meiosis more slowly than their mates. It is true that pairs were seen in which the two members were at slightly different stages. However, these were no more frequent than in the controls, and the unirradiated mates (judged by lack of crystals) were just as likely to be slower. Hence, a differential slowing of any except the very late stages of meiosis cannot be responsible for more than a small part of the X-ray effect. ELLIOT and CLARK (1955) found abnormalities in meiosis in Tetrahymena only after doses of 100 kr or greater, and this supports the conclusion that only postmeiotic stages are abnormal at lower doses.

In this connection, CHEN's cytological studies (1940, 1951) on nuclear behavior at conjugation in *Paramecium bursaria* are of importance. He figures several instances of one-sided nuclear exchange in pairs in which the two members are in the same stage of nuclear development so that there could have been little if any differential slowing. Two pairs from a cross of a young and an old clone are of special interest in the present connection. In both, the young member contributed a gamete nucleus to its older mate, but the latter retained both its gamete nuclei. The three gamete nuclei in the old member failed to fuse and developed as three separate hemikarya. Failure of fusion was also found in some pairs in which exchange was complete. Failure of exchange and failure of fusion may have been involved in the behavior of irradiated clones though cases of one-sided exchange with fusion must also have occurred. It is tempting to homologize the behavior of old clones with that of irradiated ones though more work would be required to see how close the parallel really is.

In conclusion, X-rays have marked effects on nuclear behavior during conjugation, but only a part of these effects can be accounted for by known chromosomal mecha-

nisms. The effects are persistent over at least several fissions, and there is every reason to believe that they are inherited indefinitely during vegetative reproduction. Therefore it seems probable that some other kind of persistent effect on the nucleus is involved. It is of interest in this connection that ATWOOD, VON BORSTEL and WHITING (1956) have found that a frequent class of dominant lethals, previously described by VON BORSTEL (1955) in Habrobracon, cannot be attributed to chromosome loss. It would be premature to suggest that there is any basic similarity between the two phenomena, but it is important to point out that persistent effects of radiation on the nucleus are not at present fully interpretable on classic models of chromosome loss or hypomorphic mutations.

SUMMARY

1. Crosses were made between X-irradiated and unirradiated *Paramecium aurelia*, and the behavior of nuclei during conjugation was inferred from the distribution of marker genes to the progeny.

2. Irradiation markedly influences the choice of a meiotic product to form the gamete nuclei and the production of a successfully functioning male gamete nucleus from this product, and these effects are transmitted during vegetative reproduction.

3. A comparison of irradiated diploids with unirradiated and irradiated haploids shows that the disturbances in nuclear behavior in the former can be attributed only in part to induced deficiencies. Anaphase bridges are not important factors.

4. Therefore, the action of radiation on nuclear behavior cannot be completely explained by the known effects of chromosomal aberrations. No alternative hypothesis can be presented at the present time.

5. No direct effect of irradiated cytoplasm was found on postconjugation survival in crosses involving irradiated amicronucleate lines.

6. Even when allowance is made for all possible selection, the frequency of dominant lethals in Paramecium is low. It is suggested that this is because (1) the species is relatively resistant to the deleterious effect of aneuploidy and (2), with the large number of chromosomes in the set, single breaks are relatively ineffective in producing losses.

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THE PROBLEM OF SYNTHETIC LETHALS IN DROSOPHILA MELANOGASTER

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Received April 2, 1956

THE "classical" concept of the lethal is one of a genetic condition present at a specific locus which in homozygous or hemizygous state causes death before completion of development. In contrast to this, DOBZHANSKY in 1946 described "synthetic" lethal chromosomes which he discovered in *Drosophila pseudoobscura* while studying variation presumably due to recombination between different second chromosomes. If lethal chromosomes can originate not only in consequence of mutational events at specific loci but also by synthesis through crossing over of new chromosomes, the question arises: how frequent are classical and synthetic lethals relative to each other? Data bearing on this question have been provided by several authors.

DOBZHANSKY's synthetic lethals arose from crosses of flies one of which carried a second chromosome that was normal in viability when homozygous and the other carried a second chromosome which in the homozygous condition was lethal at 25.5°C but viable at 16.5°C. Three of the resultant chromosomes were lethal to homozygotes at 16.5° as well as at 25.5°. These new chromosomes were interpreted by DOBZHANSKY as being recombinants of the two original chromosomes. Crossing over, it was assumed, had brought together complementary loci or regions which separately, permitted survival at 16.5°, but which together, in homozygous state, produced lethality.

In 1949 MISRO published results of experiments to test for lethality due to recombination between pairs of normal chromosomes in *D. melanogaster*. Flies from wild populations were used, females heterozygous for two different wild type second chromosomes serving as the source of recombinants. The controls with recombination either absent or irrelevant were males heterozygous for the same two chromosomes and females homozygous for each wild type chromosome. MISRO found that lethals appeared about four times as frequently in heterozygous females as in homozygous females. This statistically significant difference was attributed to recombinational lethals.

WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE, (1953) testing variability arising through recombination of second chromosomes in *D. melanogaster*, found that lethals arose with higher frequency in the females heterozygous for two different wild type second chromosomes than in the controls which were homozygous for each of the chromosomes. This difference in the lethal frequency was again explained as being due to recombinational lethals. Twenty-eight combinations of the 45 heterozygotes produced lethals. The total frequency of lethals in all 45 was 2.96%. The control frequency was 1.20% in the nine homozygous classes tested.

According to WALLACE *et al.*, "selection favors particular heterozygotes and thereby

builds up selected series of alleles at all (or at least many) loci—the particular alleles at any locus being determined in each population by those present at all other loci." It is this heterozygosity that accounts for variability when crosses are made between different chromosomes and may in extreme cases form lethal chromosomes. This is contrasted with the theory that selection always favors a particular allele at a particular locus, thus selecting for homozygosity in a population.

The following report deals with tests for synthetic lethals in the X and third chromosomes of *Drosophila melanogaster*.

X CHROMOSOME LETHALS

In 1955 it was reported (HILDRETH) that tests for synthetic lethals in the X chromosome of *D. melanogaster* produced results in contrast to those of the abovementioned authors. Ten different wild type laboratory stocks were used for the experiment with the ten homozygous classes serving as controls for the 45 possible heterozygous classes. One lethal was found in the 994 control chromosomes tested and 13 were found in the 5186 chromosomes from the recombination line. Two of the 13 lethals were allelic and arose in one culture, indicating an earlier nonrecombinational origin of these two lethals. The difference between the lethal frequencies of the control and experimental groups was not significant, indicating that recombination had a small role, if any, in the increase of the lethal rate in the experimental group. When the 1955 report was published, complete localization tests on the lethals had not been carried out; therefore the results of the localizations are presented here.

Even though the results did not require the assumption that a substantial fraction of the lethals arose by recombination, there remains the possibility that some of the lethals may be recombinational ones. This can be tested by genetic localization of the lethals. If a lethal is at a specific locus on a chromosome, localization tests with different markers will give consistent results. If, however, the lethal is due not to one particular locus or gene but to the interaction of two or more specific alleles of different genes, then nonlethality will be restored to the chromosome by any crossing over that removes any of these alleles from the lethal combination. This will confuse the genetic localization analysis since separate tests with different markers may place the lethal locus at a different region in each test. In a test of a female containing a single locus lethal, theoretically 50% of the chromosomes carry the lethal. With a recombinational lethal the nonlethal chromosomes will be more frequent owing to crossing over between the different loci that interact to form the lethals. Of course, the closer the loci are, the more difficult it will be to tell such a lethal from one that arose at only one locus. However, if the loci are very close together then the frequency of the origin of such a lethal will be low.

All lethals of the X chromosome, with one exception, were tested, each with two different groups of markers and the localization tests showed no discrepancy that would necessitate an explanation of lethality due to interaction of a combination of loci. The exception was one lethal which was lethal in the tests but became semilethal in stock; i.e., males carrying this mutant occasionally survived. This lethal was not localized; since the survivors had no distinguishing phenotype. One other lethal (L# 3) at first gave strange results because of occasional survivors. However, further

TABLE 1

X chromosome lethals from heterozygous females as localized in individual tests with markers shown

Lethal no.	Class	Markers used in localizations				
		y cv v f	*y rb*	*y sn v*	*g sd B*	*f car*
1	Sal/Fres*					
2	Sal/Fres	65.6				65.7
3	Sal/Cor	48.0			49.6	52.2
5	Sal/Ore	67.4				63.4
9	Sam/Tem	50.2			50.7	
11A	Sam/Ore**	56.7			56.8	
B	Sam/Ore**	56.3				
12	Sam/Cant	49.6			50.2	
13A	Fla/Cor	0.0	0.0			
B	Fla/Cor	23.5		21.0		
14	Fla/Sac	56.7			56.5	
15	Sal/Fla	0.3	0.0			
16	Sal/Ore	61.2				62.5

* Semilethal, not tested.

** Allelic.

localization tests were made and the results were compatible with the theory that the lethal was at a single locus on the chromosome. The lethals and their loci as determined by separate tests are given in table 1. The lethals were kept in stock balanced with the "Binscy" (*y sc*^{8l} *B In49 sc*^{8}) chromosome so there would be little chance of losing a lethal by crossing over especially if the lethals were recombinational lethals.

THIRD CHROMOSOME LETHALS

Because the tests with X chromosomes indicated that recombination played no role in the production of lethals, it was decided to study the same problem for the third chromosome.

Material and method

Eight different stocks were used for the third chromosome experiments. Three of these were stocks that had been kept for several years in the laboratory. These three were cultures from which X chromosomes had been isolated and tested and they were chosen for this reason. They include a stock from Formosa (For); one from Salta, Argentina (Sal); and an inbred stock, Samarkand (Sam). To minimize the possibility of selection for specific genotypes in the course of laboratory culturing five wild type stocks from nature were also chosen for the experiments. One of the freshly caught wild types (A) was trapped in Berkeley, California in October 1954. The second and third (B and C respectively) were trapped at different localities, separated by more than two miles, in Orinda, in October and November 1954. The fourth (E) was trapped at Riverside in December 1954, and the fifth (F) was trapped in September 1954 in Napa. A crossover suppressor chromosome was needed and for this the *DcxF* chromosome was chosen. This will be indicated by *D* in the crosses. The

experiments to determine the frequency of lethals produced in recombinational and nonrecombinational series were started in July 1954 and terminated in July 1955.

From each of the eight stocks one nonlethal third chromosome was isolated and all the chromosomes in the control and recombinational tests were descended from these. Salivary chromosome analyses did not reveal any detectable chromosomal aberrations. It is important that there be no obvious aberrations, since crossing over should be normal. Each chromosome was isolated by mating a wild type male to virgin *D/Sb* females. One *D/+* male from the F_1 generation was mated with virgin *D/Sb* females.

Among the offspring of this mating would be males and females of the constitution *D/+* whose wild type third chromosomes were all derived from a single sperm in the P_1 male. Matings of these *D/+* males and females would produce wild type (+/+) offspring if the original + chromosome was not lethal. From cultures in which +/+ flies appeared stocks were obtained in which the wild type chromosomes were kept balanced with the *DcxF* chromosome. Twelve generations elapsed between trapping of the fly bearing the first of the third chromosomes tested (A) and the mating of the last heterozygote (A/F) bearing this initial chromosome. Three more generations elapsed between the mating of the fourth and fifth wild types, the last in the series.

The experimental procedure for determination of lethals is outlined below. Since the experiments were carried out over a period of weeks it was necessary to check for spontaneous lethals that might arise in the stocks and needlessly complicate the results. The outline below assumes that each chromosome of cross G1 was not carrying a lethal. The experiment was also set up so that heterozygous males (G2), serving as noncrossover controls, would be brothers of the heterozygous females thus making the genotypes of the two as nearly identical as possible. Throughout the crosses, except in the heterozygous wild type females, recombination between the third chromosomes of females was minimized by the crossover suppressor *DcxF* chromosome. The outline of the experimental procedure uses the Formosa (For) and Salta (Sal) chromosomes as an example.

G1:	1 *D*/Sal ♀	×	1 *D*/For ♂
	Experimental Series of Crosses		Control Series of Crosses
G2:	4(1 Sal/For ♀ × 2 *D/Sb* ♂♂)		4(2 *D/Sb* ♀♀ × 1 Sal/For ♂)
G3:	100(2 *D/Sb* ♀♀ × 1 *D*/+ ♂)		100(2 *D/Sb* ♀♀ × 1 D/+ ♂)
G4:	100(2 *D*/+ ♀♀ × 2 *D*/+ ♂♂)		100(2 *D*/+ ♀♀ × 2 *D*/+ ♂♂)
G5:	*D*/+ and +/+		*D*/+ and +/+

In the experimental series some of the +/+ flies will be homozygous for recombinant chromosomes while in the control series the individuals will carry only noncrossover chromosomes. It is the recombinants which may account for the production of possible synthetic lethals.

All experiments were carried out at 26°. The G5 cultures were determined to be lethal or nonlethal containing 15 days after the cultures were started. Under ideal conditions one would expect approximately two thirds of the offspring to be Dichaete and one third normal wild type since Dichaete is lethal in homozygous condition. In the tests for lethality a minimum number of *D*/+ flies per culture with no +/+ flies present had to be set as a standard. If there were at least the minimum number and

no +/+ flies, the culture was to be scored as a lethal. If there was not the minimum number and there were no +/+ flies the culture was scored as doubtful. The presence of one or more +/+ flies, no matter what the total number of flies in the culture, would mean the culture was to be scored as nonlethal bearing. The minimum number per culture was set at 20 Dichaete flies and none wild type. Of the 20 flies, all could be offspring or one to four could be parents since the parents were not removed from the cultures. A χ^2 test, using the maximum (20) and the minimum (16) when one third of the flies are expected to be wild type (13.7 Dichaete to 6.3 wild type and 10.7 Dichaete to 5.3 wild type, respectively) indicates that such results would be found by chance less than once in 100 times (χ^2 = 9.19 and 7.99 respectively). If there were at least 20 flies present and none was wild type, the culture was assumed to be carrying a recessive lethal in the + chromosome. Three new creamers were then started from this culture, each with three pairs of *D*/+ flies. Fifteen days later these cultures were examined for the presence of wild type flies. If any were present (one or more) in any creamer the original culture was declared not lethal. If there were no wild type flies in any creamer and there were more than 50 flies in at least one creamer, then the culture was scored as lethal. If there were less than 50 flies the culture would be scored as doubtful and three creamers would be started again. However, in these experiments no cultures had to be tested a third time. The minimum of 50 *D*/+ flies with no +/+ flies present was chosen to eliminate as much as possible the scoring of semilethals as lethals. The probability of finding a culture with 50 *D*/+ and no +/+ flies when the expected ratio is 33.7 to 16.3 would be much less than one in 100 with a χ^2 value of 24.18. (Six of the 50 flies may be parent flies. In this case one would expect 29.3 Dichaete to 14.7 wild type flies. The probability of obtaining such a culture by chance alone would still be much less than once in 100 times.) When lethals were found in the second check, cultures were started keeping the lethals balanced with the *DcxF* chromosome.

If in the first check a culture had less than 20 flies and none was wild type the culture was labelled "doubtful" and either new matings of the *D*/+ sibs were made or the original parents were transferred to a fresh creamer. These were again examined 15 days later. Only six "doubtful" cultures occurred, and all were found to belong to the nonlethal class on the second check.

Only five cultures were sterile. In each case their sterility was due to early death of the G4 flies.

Lethal frequency

The results of the tests for lethal frequency are presented in table 2. In the table three groups (For/A, A/F, E/F) marked with daggers produced several lethals (8, 8, and 9, respectively). The lethals within each group were found to be allelic by crossing together and their occurrence can best be explained as due to descent from a single nonrecombinational lethal arising in an earlier generation.

The total number of lethals found was 15 out of 2771 chromosomes tested in the recombinational line, and 18 out of 2693 chromosomes in the nonrecombinational control line. From these raw data there is no reason to believe that recombination plays any role in producing lethals since the frequency is higher in the control line.

TABLE 2

Frequency of lethals

Classes indicate combinations of wild type third chromosomes. Numbers in columns indicate chromosomes tested. Superscripts indicate numbers of lethals found among chromosomes tested. Where superscript is absent no lethals appeared. Fractions show total lethals over total chromosomes tested. Roman numerals indicate G2 females or males. Within any class each row represents a separate experiment which lists the G2 flies derived from a single pair of G1 individuals. (Those marked * are probably premeiotic clusters arising in the G2 germ lines; † probably prerecombinational lethals and not included in totals.)

Class	Females							Males					
	I	II	III	IV	V	VI	Total	I	II	III	IV	V	Total
For/Sal	21	9	16	14	14^{1}	21		21	22	32	22		
	15	9	12	33				26	18	15			
	13^{1}	17	11	17			2/222	16^{4*}	18	22			4/212
Sam/For	10	13	25	17	29			39	21	25			
	31	30	25					19	31^{1}	28	19		
	11	17	22	13	14		0/257	13	10	15	13	16	1/249
Sam/Sal	14	19	11	31	6	12		24	15	27	20	12	
	29	22^{1}	27	23			1/194	15	32	22	16	24	0/207
Sal/A	29	20	10	15	10			18	14	19	10	23	
	21	21	24	33			0/183	28	21	14	37		0/184
For/A	19^{1}	28	13	22	23			30	23	28	14		
	31	34	18^{8}†	19			1/189	20	29	20	27		0/191
A/B	30	23	21	32				29	16	34	18	9	
	17	32	30	27			0/212	26	18	43^{1}	22		1/215
A/C	36^{1}	39	30^{4*}					25^{1}	16	18	28	11	
	23	35^{1}	20^{1}	32^{1}			8/215	31	15	24	30^{1}		2/198
B/C	13	20	21	14	14			16	22^{1}	19	20		
	20	18	16	15	22		0/173	15	21	21	24	23	1/181
A/E	13	12	20	29	22			28	25	9	20		
	25	26	34				0/181	19	30	15	24^{1}		1/170
B/E	23	21	28	21				20	24	20	16^{5*}		
	12	22	14	31^{1}	10		1/182	22	21^{2*}	27	14		7/164
C/E	20	24	36	20				22	28	31			
	35	22	31				0/188	37	25	35			0/178
A/F	19	13	21^{1}	16	23			21	25	18	19^{3}†		
	25	27	21	21			1/186	21	31	18			0/134

TABLE 2.—*Cont.*

Class	Females							Males					
	I	II	III	IV	V	VI	Total	I	II	III	IV	V	Total
B/F	10	15	18					24	8	18			
	19	21	8	14			0/105	19	17	16	16		0/118
C/F	13	8	23	26				29	24	15			
	14	37	19	9			0/149	20	20[1]	23	28		1/159
E/F	17	11	15	27	20			16	15[9]†	19	23	14	
	10	12	5	5[1]	5	8	1/135	12	9	17	11	12	0/133
Total Lethals							15/2771						18/2693

However, it is necessary to consider a source of error other than the presence of prerecombinational lethals in the heterozygous wild type experimental females or control males of G2. This is the possible occurrence of premeiotic lethals arising in the germ line of these G2 females and males. Such lethals would give rise to clusters of lethals, the number within each cluster being dependent upon the stage in development of the germ tract when the lethal arose and upon the sampling technique (SPENCER and STERN 1948; CASPARI and STERN 1948).

In table 2 the clusters of lethals are marked with an asterisk. All lethals within each cluster were tested by intercrosses and found to be allelic in each case, indicating that common origin of the lethals was possible. Since it will be shown below that all lethals formed must be regarded as having arisen by mutation, not by recombination, the allelism within clusters means either a single common origin or that several mutations occurred at the same loci. The latter occurrence is extremely unlikely.

If each cluster is considered as one lethal the number of lethals in the recombination line will drop from 15 (.541%) to 12 (.433%) in 2771 while the control will decrease from 18 (.668%) to 10 (.371%) in 2693 chromosomes tested. It is not entirely accurate to correct in this way since some of the nonlethals have arisen from single gonial cells which then form nonlethal clusters, but the error has the same effect on experimental and control groups.

In the study of recombinational lethals we are ideally concerned only with those lethals which arise as a result of crossing over in meiosis. In addition, we must expect lethals which arise independently of crossing over and occur either as singles or in clusters. The experimental lethal frequency should then include the normal spontaneous lethals plus those which became lethal during meiosis as a result of recombination. Probably the best estimate of lethal frequency in our case would be to eliminate all clusters from the calculations despite the inaccuracies involved. If this is done the experimental values listed in table 2 will be lowered to 11 lethals in 2741 (.401%) and the controls to seven lethals in 2640 (.265%) chromosomes tested. These values are not significantly different (P value = .5 − .3) using a χ^2 test. Another method of treating these results would be to test for significance of difference between the number of mothers producing lethals in the experimentals and the number of

TABLE 3

Lethal frequency of experimentals (female) and controls (males) from tests on third chromosome. A = total lethals; B = each cluster of lethals counted as one lethal; C = all groups containing clusters of lethals discarded from totals. In the fractions the numerator is the number of lethals and the denominator the number of chromosomes tested. LW = laboratory wild type stocks and FW = fresh caught wild type. Figures in parentheses are percent lethal

	A		B		C	
	Females	Males	Females	Males	Females	Males
LW × LW	3/673 (.445)	5/668 (.748)	3/673 (.445)	2/668 (.299)	3/673 (.445)	1/652 (.153)
LW × FW	1/372 (.269)	0/375 (0.0)	1/372 (.269)	0/375 (0.0)	1/372 (.269)	0/375 (0.0)
FW × FW	11/1726 (.637)	13/1650 (.788)	8/1726 (.463)	8/1650 (.485)	7/1696 (.413)	6/1613 (.372)
Total	15/2771 (.541)	18/2693 (.668)	12/2771 (.433)	10/2693 (.371)	11/2741 (.401)	7/2640 (.265)

fathers producing lethals in the controls. In the experimental series 11 mothers out of 137 produced single lethals while in the controls 7 fathers out of 124 produced lethals. A χ^2 test of these data gives a P value of .5 − .3. If we used either of the other methods of including lethals, (table 3) 15 in the female line to 18 in the male line (A) or 12 in the female line to 10 in the male line (B), the results would be still less compatible with the hypothesis that lethals often are formed by recombination. Table 3 compares the number of lethals if (A) all lethals are included, or (B) a cluster is counted as one lethal, or (C) clusters are eliminated from the analysis. This table also compares the results with flies from laboratory stocks and flies freshly caught in nature. The samples are small but no evidence appears in favor of a higher frequency of lethal formation in the freshly caught wild types due to recombination.

Localization of lethals

The lack of statistical significance of the difference in frequency of lethals between the experimental and control tests for recombinational lethals in the third chromosome of *D. melanogaster* does not necessarily mean that none of the lethals in the experimental group could have been formed by crossing over. The possibility remains that some of these lethals derived from the heterozygous females could have been produced by recombination between the third chromosomes.

Genetic localization tests were conducted on the third chromosome lethals derived from the heterozygous females. Two separate tests were carried out for each lethal but one, using different markers in each test. One of the lethals became semilethal in the stock bottles and was not tested for its location on the chromosome. The lethals were kept balanced with the *DcxF* chromosome so that there was little opportunity for crossing over to restore normality to a lethal chromosome. The method of localizing the lethals is more complicated and more time- and space-consuming than the method used for X chromosome lethals. The method used in the third chromosome

tests is outlined below:

$$G1: 1\ \frac{D}{\text{lethal}}\ ♀ \qquad \times 2\ \frac{h\ st\ sr\ ca}{h\ st\ sr\ ca}\ ♂♂$$

$$G2: 4\left(1\ \frac{\text{lethal}}{h\ st\ sr\ ca}\ ♀ \qquad \times 5\ \frac{D}{\text{lethal}}\ ♂♂\right)$$

$$G3: \pm 100\left(2\ \frac{h\ st\ sr\ ca}{h\ st\ sr\ ca}\ ♀♀ \times 1\ \frac{\times -\text{over?}}{\text{lethal}}\ ♂\right)$$

It is in the germ cells of the heterozygous females in generation G2 that crossing over between the lethal-bearing and marked chromosomes will occur. The progeny of the G2 cross will be phenotypically Dichaete and wild type in the ratio of 2 to 1 respectively. All wild type males were taken from each bottle and mated singly in creamers with two virgin *h st sr ca* females. In any one creamer the offspring of the G3 cross will be 50% *h st sr ca*/lethal (wild type) and 50% will be the crossover or noncrossover chromosome over the marked chromosome. If a culture contained *h st sr ca* offspring in addition to wild type offspring, then the test chromosome was a noncrossover chromosome. If a culture contained all wild type offspring, the test chromosome was wild type due to crossing over. If any of the flies in a creamer were any specific combination of three or fewer of the recessives the test chromosome would be a specific cross over type and would be scored as such.

In this way all chromosomes were tested and scored. Each lethal behaved as a single-locus lethal and it was not necessary to assume that any had arisen due to crossing over between chromosomes. Table 4 lists the lethals and their loci as established by two separate tests for each lethal.

TABLE 4

Third chromosome lethals from heterozygous females as localized with markers shown

Lethal no.	Class	Markers used				
		h st sr ca	*ru h*	*jv se by*	*h st p^p ss*	*st p^p ss e^s*
1	Sal/For	46.8			±48*	
2	Sal/For**					
5	Sal/Sam	48.5			48.0	
6	For/A	51.7			±53*	
9	A/C	45.7			46.0	
12	A/C	47.0			48.0	
13	A/C	63.9				69.1
14	A/C	44.0			45.6	
17	B/E	10.8	10.8			
21	A/F	33.1		33.1		
23	E/F	44.0			44.0	

* Test not large enough to localize lethal more exactly. Crossing over occurred on one side of lethal only. Localizations compatible with one locus lethal theory.

** Semilethal, not tested.

DISCUSSION

The results of the work reported here on the X chromosome and the third chromosome in *D. melanogaster* are different from those of other investigators who studied the second chromosome of several other species. Some of the lethals studied in the second chromosome of *D. melanogaster* and in other species are interpreted by the authors as arising by recombination of alleles of different loci which by themselves are not lethal. Lethals evidently are not formed in this way in the X chromosome of *D. melanogaster*. At least neither frequencies nor localization tests gave any indication that lethals arose in this manner in those chromosomes which were tested.

It is possible that the third and X chromosomes behave intrinsically differently from the second chromosome with respect to recombinational lethals. There are, however, no obvious reasons for such differences. Particularly the third and second chromosomes, which are both autosomes and of similar length, would *a priori* not be expected to give opposite results. The X chromosome would be under more rigorous selection pressure than an autosome owing to its appearance in the hemizygous condition in the male, and it might seem that this could be a cause for difference between the second and X chromosomes. However, if it is true that selection favors heterozygosity then one might expect that even in the X chromosome heterozygosity might be favored. The work of KERR and KERR (1952) has shown that there are viability differences among different X chromosomes of *D. melanogaster* and that there are viability differences between the sexes when the same hemizygous X chromosome of the male is made homozygous in the female. This indicates that there is heterozygosity in the X chromosome and that there are different alleles of different loci involved, and perhaps in various combinations. It is, of course, possible that crossing over in the X chromosome does not produce lethals because of the more rigorous selection in hemizygotes permitting heterozygosity to give rise to viable X's only, since frequent occurrence of lethals in the X chromosome would be detrimental to the survival of the species.

Another possible reason for the difference between the X chromosome and second chromosome results could be that the X chromosomes used came from populations that had been in laboratory culture for at least three years when the experiments were started, as compared with the situation in the second chromosome where WALLACE selected 10 chromosomes from large experimental populations. Because each stock was kept in half-pint culture bottles, selection could have worked in such a way that all X chromosomes had become relatively similar to one another, in spite of the fact that they may have been greatly different when the flies were first trapped in various parts of the world. If this were true, then recombination might not produce lethals since the recombinant chromosomes might not be sufficiently different from the noncrossover chromosomes except for possible mutations. But if this explanation for the absence of synthetic lethals in the X chromosome were correct, then it should also hold for the third chromosome. Yet, the third chromosome data show no great difference between the frequencies in the chromosomes from laboratory stocks and in chromosomes from the freshly caught wild types. There is then no ground for assuming that selection within the laboratory stocks has eliminated an otherwise present recombinational effect. Still another reason for the divergent results could

be that in the experiments described here chance alone was responsible for the fact that no chromosomes were selected which in recombination could give lethals. This again seems unlikely since there were 45 heterozygous combinations for the X chromosomes and 11 combinations heterozygous for the third chromosomes. It would not seem probable that chance alone would be responsible for MISRO's obtaining three wild type chromosomes and having the two combinations which he studied produce synthetic lethals if none of the larger samplings of the third and X chromosomes produce them. Nor does it seem likely from chance alone that 28 of WALLACE's 45 combinations should produce lethals by recombination if none appears from the 56 combinations of the X and third chromosomes.

The major difference between DOBZHANSKY's work with *D. pseudoobscura* and that reported here is that one of the *pseudoobscura* chromosomes was initially strikingly subnormal in effect whereas the present studies reported herein began exclusively with "normal" chromosomes (table 5). The *pseudoobscura* chromosome was lethal at 25.5°C in the homozygous state but permitted normal survival at 16.5°. The synthetic lethals obtained were lethal at 16.5°C as well as at the higher temperature. The origin of these lethals was interpreted as resulting from crossing over between the two parent chromosomes.

A similar difference is in part involved between the second chromosomes used by WALLACE *et al.* and the X and third chromosomes. In WALLACE's work four of the ten chromosomes were specifically chosen because of their poor viability in the homozygous condition. One of these gave 6.5% lethals in the homozygous condition. This is a higher frequency than found in any of the heterozygous combinations of the third chromosome. Combinations involving these four chromosomes are responsible for the majority of the lethals observed. One hundred and twenty-two lethals in 3235 tested chromosomes (3.77%) were observed in the groups which were combinations of the four chromosomes having poor viability. It is likely that recombination between the chromosomes involved in these experiments was the source of some of the synthetic lethals observed by WALLACE *et al.* On the other hand, combinations of the chromosomes that had high viability yielded similar frequencies of lethals from homozygous controls and heterozygous experimentals (.49% and 1.31%

TABLE 5

Viability of flies bearing third chromosomes homozygous for each wild type chromosome tested. Results are from crosses of D/+ *females with* D/+ *males. Expected wild type frequency is 33.33%*

Class	No. of cultures	Offspring		Total	% wild type
		D/+	+/+		
A	6	299	198	497	39.84
B	6	241	204	445	45.84
C	6	348	203	551	36.84
E	6	263	155	418	37.08
F	4	148	124	272	45.59
Sal	5	300	162	462	35.06
For	5	324	186	510	36.47
Sam	6	342	217	559	38.82

respectively). A χ^2 test gives a P value of $.3 - .2$, indicating that chance alone could be responsible for the observed differences in lethal frequency.

MISRO's results from studies of lethality in the second chromosome in *D. melanogaster* were reported in abstract form and few details are given. This makes it impossible to analyze his results adequately and to compare them with those on the X and third chromosomes.

WIGAN (1949) localized the second chromosome lethals that arose in MISRO's experiments as well as some from his own experiments and found that the synthetic lethals differed from the "point" lethals in their distribution along the chromosome. Of the 23 synthetic lethals tested nine were in the left arm between 15.0 and 45.0, seven in the right arm between 70.0 and 95.0, one near the centromere and one near the left end and five seemed to lie beyond the right end of the chromosome. Of the point lethals three were in the left arm, nine in the right, and five near the centromere. Eighteen lethals were collected from wild populations and these had a distribution similar to that of the point lethals mentioned above. He concluded that even though synthetic lethals may occur in wild populations they may not be found often because of their unstable nature. Contrary to this, PAVAN and KNAPP have suggested that the high frequency of lethals found in their sample of *D. willistoni* populations might be due to synthetic lethals.

The results presented here for the X chromosome (table 1) and the third chromosome (table 4) have a distribution similar to that of WIGAN's point lethals. In the X chromosome eight lethals are within approximately 16 units from the centromere (66.0), two at the left end, and one at 21.0. In the third chromosome seven lethals are not more than seven units to the right or left of the centromere (46.0), with two others in the left arm and one in the right arm.

A further source of difference could be in the culture technique used and also in the method of determining lethality. The authors mentioned determined lethality upon the first check of a culture and if at least the minimum number of flies was present and there were no wild type flies the culture was classified as lethal. For this work a more rigorous method was used in that each lethal was tested a second time and the culture was classified as lethal or nonlethal depending on the results of the second check. Only three cultures in the recombinational line and four in the control line became nonlethal on the second check. These would have had little effect on the total results. If localization tests had been done on these "first-check lethals" the analysis would have been confused by the emergence of the unexpected and supposedly absent genotypes of a true lethal.

The divergent results of experiments to determine whether lethals arise as a result of recombination of specific alleles of loci which in their original combination were not lethal may not be as opposed to each other as it might seem. When the results of the experiments are compared one must not lose sight of the intrinsic differences of the chromosomes used by the different authors. It is known that chromosomes in nature range in viability from those that are lethal in the homozygous state to those with normal viability. If any of those are chosen which already carry a recessive lethal or have factors causing poor viability it may not seem so strange that recombinational lethals be produced. On the other hand if chromosomes with high viability are

recombined by crossing over, synthetic lethals may be rare. This has been shown in the work described here on the third and X chromosomes. Thus, synthetic lethals may arise frequently in natural populations, but the phenomenon may not be a universal one. That is, recombinational lethals need not necessarily be expected to arise frequently as a result of crossing over even though the chromosomes involved may be from different populations.

SUMMARY AND CONCLUSIONS

Experiments were carried out on *Drosophila melanogaster* to determine if lethals are formed by recombination between normal X chromosomes of different populations and between normal third chromosomes of different populations.

Statistically the results are in agreement with the hypothesis that recombination played no role in producing lethals in the X chromosome or in the third chromosomes studied. Genetic localization tests, using different sets of markers in at least two separate tests for each lethal, indicate that there is no need to assume that a combination of loci or of regions is involved in any of the lethals.

The results of these experiments on the X chromosome and the third chromosome are different from those of other authors who have interpreted their findings as showing that lethals frequently are produced by recombination in the second chromosome of several Drosophila species. The production of synthetic lethals in the second chromosome of those Drosophila species studied may be due to synthesis of recombinant chromosomes from chromosomes which were originally subnormal in effect.

Crossing over is perhaps a factor in the production of lethals in natural populations, but experiments on the X and third chromosomes indicate that lethality need not be expected to occur frequently as a result of crossing over, especially if the parent chromosomes are free from pre-existing lethal or severe viability defects.

ACKNOWLEDGMENTS

The author wishes to thank his wife, Gretchen, for assistance in typing and editing this manuscript. It is also a pleasure to thank DR. CURT STERN for his interest and advice throughout the course of the experimental work and preparation of the manuscript.

This work was supported by the U. S. Atomic Energy Commission.

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PHASE VARIATION IN SALMONELLA[1]

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Received April 8, 1956

THE flagella of most Salmonella serotypes occur in two alternative, clonally stable phases. Earlier applications of transductional technique have shown that the phases correspond to two loci, H_1 and H_2 (LEDERBERG and EDWARDS 1953). The present study is concerned with the internal control of the alternation.

1. HISTORICAL BACKGROUND

1.1 Serological analysis of Salmonella dates to the differentiation of antigenic components in motile and nonmotile strains of *S. cholerae-suis* by SMITH and REAGH (1903). They and subsequent authors have classified these components as an "H" thermolabile, flagellar and an "O" thermostable, somatic antigen respectively. Further analysis has proceeded by the now familiar techniques of cross-absorption of antiserums prepared against various strains, and has led to the elaborate tabulation known as the KAUFFMANN-WHITE diagnostic scheme, part of which is abstracted in table 1. The scheme is an important guide in epidemiological tracing of Salmonella infections, and newly discovered types are therefore promptly recorded and deposited with International Salmonella Typing Centers. The cooperative efforts of hundreds of bacteriologists therefore make a wealth of information and natural variants available for genetic study.

1.2 The most striking feature of the scheme is its combinatorial pattern—*prima facie* evidence for a mechanism of genetic recombination which encouraged eventually successful efforts at its demonstration (LEDERBERG 1947, 1948; ZINDER and LEDERBERG 1952). The serotypes entered in the table can be construed as generated by the combination of three basic elements, the somatic antigen complex and the two flagellar phases. As we will not be immediately concerned with the former it will be symbolized only by the conventional groups, rather than detailed formulae. However, such abbreviations denote antigenic patterns whose genetic interest is no less than that of the flagellar factors (cf. UETAKE *et al.* 1955).

1.3 Flagellar phase variation was discovered by ANDREWES (1922) in *S. typhimurium*. Mass cultures of this serotype may agglutinate in either of two H-antisera, anti-i or anti-1.2. A reisolated clone might react only, say, with i. On further cultivation, the i clone will generate an occasional offshoot which reacts only with 1.2. In its turn, the 1.2 phase is liable to throw off an occasional i, an alternation which will be symbolized i:1.2. The variation from one phase to another may occur at any

[1] Paper No. 618 of the Department of Genetics. This work has been supported by research grants from the National Science Foundation, from the National Cancer Institute (C-2157), Public Health Service, and from the Research Committee, Graduate School, University of Wisconsin with funds allocated by the Wisconsin Alumni Research Foundation. The numbering of paragraphs in this paper is intended to facilitate internal cross-reference and should otherwise be ignored.

time, with a small probability per bacterial division, which ranged from 10^{-5} to 10^{-3} in the strains studied by STOCKER (1949). In rapidly varying strains, phase variation can be detected by random colony tests; in more stable material, selection by the immobilizing action of antiserum against the existing phase is effectively practiced.

1.4 While its stochastic indeterminacy tends to signify phase variation as mutation, it is bounded by two fixed alternatives which are characteristic of the serotype. As shown in table 1, any phase-1 antigen may be associated with any phase-2, and the one cannot be predicted from the other. The table also shows that the flagellar antigens can be divided into two groups, viz., phase-1: a, b, c, d, eh, i, r, etc., and phase-2: 1.2, 1.5, 1.7, enx_x etc. One antigenic alternative is chosen from one group, one from the second. This pattern of variation has no parallel in the mutation of other genes, and until recombinational analysis was applicable, genetic interpretations of phase variation could only be speculative (DUBOS 1945; LEDERBERG 1948).

1.5 The genetic relationship of the phases in a wide variety of serotypes has been investigated by transductional techniques (LEDERBERG and EDWARDS 1953). For example, the transduction from *S. typhimurium*, i:1.2, as donor, to *S. abony*, b:enx as recipient gave the new combinations i:enx and b:1.2, but not such types as i:b or

TABLE 1

The KAUFFMANN-WHITE scheme of Salmonella serotypes, condensed from KAUFFMANN (1951)

Salmonella serotype	Somatic antigen	Flagellar phase-1	Antigens phase-2	Salmonella serotype	Somatic antigen	Flagellar phase-1	Antigens phase-2
kisangani	B	a	1.2	manhattan	C2	d	1.5
arechavaleta	B	a	1.7	newport	C2	eh	1.2
bispebjerg	B	a	enx	kottbus	C2	eh	1.5
tinda	B	a	enz_{15}	takoradi	C2	i	1.5
abortus-equi	B	—	enx	bonariensis	C2	i	enx
paratyphi B	B	b	1.2	bovis-morbificans	C2	r	1.5
abony	B	b	enx	hidalgo	C2	r	enz_{15}
wagenia	B	b	enz_{15}	sendai	D	a	1.5
altendorf	B	c	1.7	loma-linda	D	a	enx
stanley	B	d	1.2	onarimon	D	b	1.2
saint-paul	B	eh	1.2	typhi	D	d	—
chester	B	eh	enx	ndolo	D	d	1.5
essen	B	gm	—	eastbourne	D	eh	1.5
typhimurium	B	i	1.2	enteritidis	D	gm	—
heidelberg	B	r	1.2	shoreditch	D	r	enz_{15}
san juan	C1	a	1.5	butantan	E1	b	1.5
oslo	C1	a	enx	shangani	E1	d	1.5
edinburg	C1	b	1.5	elisabethville	E1	r	1.7
cholerae-suis	C1	c	1.5	marseille	F	a	1.5
mission	C1	d	1.5	chandans	F	d	enx
lomita	C1	eh	1.5	aberdeen	F	i	1.2
montevideo	C1	gms	—	veneziana	F	i	enx
virchow	C1	r	1.2	mississippi	G	b	1.5
narashino	C2	a	enx	heves	H	d	1.5
gatuni	C2	b	enx	hvittingfoss	I	b	enx
muenchen	C2	d	1.2				

1.2:enx, which would have been as readily detectible by the selective method. These and many analogous results imply that the phase-1 antigens form one genetic homology group, the phase-2 a second. The factors within each group are mutually interchangeable by transduction, while factors of different groups are not.

1.6 In a word, each group is the set of alleles of an H_1 and H_2 locus, respectively, so that the phenotype b:enx corresponds to the genotype $H_1^b H_2^{enx}$. Furthermore the two loci are independently transduced, i.e., they are not closely linked to one another for the i:1.2 class was also not produced. If the two antigenic phases are thus determined at separate loci, phase variation cannot be construed as the mutation from one specific allele to another. Instead, it is the alternative manifestation of each of the two antigenic specificities already inherent in the genotype. This concept was already provoked by the closed cycle of variation emphasized above.

1.7 To investigate this alternation, experiments were directed primarily at the inheritance of flagellar phase in recombinant clones generated by transduction. Where the phase of a culture was determined, it will be indicated by bold face: thus, b:**enx** signifies a clone displaying the enx antigen that varies to the **b**:enx form, in which the b antigen is now manifest.

1.8 Recombinational analysis in Salmonella (so far) depends on a system of transduction (the transmission of hereditary fragments) mediated in this instance by bacteriophage particles (ZINDER and LEDERBERG 1952). The leading work on this system involved a variety of nutritional, fermentative and resistance markers, and was followed by studies on mutants affecting the formation and action of flagella (STOCKER *et al.* 1953) and on the flagellar antigens (LEDERBERG and EDWARDS 1953). Every marker that was studied and was technically feasible has been subject to transduction at a more or less uniform incidence (cf. ZINDER 1955, for quantitative comparisons of transduction efficiency of different factors). The symbolism A —x B or B x— A will be used to indicate a transduction with A as donor, B as recipient, i.e., the application of a cell-free lysate of A to intact cells of B.

2. MATERIALS AND METHODS

2.1 Transduction was carried out with lysates of Salmonella phage PLT22 grown on indicated host cultures as previously described, 1.8

2.2 Two modes of selection were used to isolate the minority of transduction clones from the excess background of unaltered recipient cells. Both methods take advantage of a soft nutrient gelatin agar (NGA) through which motile bacteria readily swarm, while nonmotile or immobilized cells grow in place. (A), Mixtures of bacteria and phage were inoculated on NGA plates to which antiserum for both phases of the recipient bacteria was added. These are therefore immobilized, but any bacteria which manifest a different H antigen, viz., one transduced from the donor, swarm from the point of inoculation. For example, outgrowing swarms of **b**:1.2 and i:**enx** from b:enx —x i:1.2 can be picked twelve to twenty-four hours after NGA plates containing i and 1.2 antiserum are inoculated. (B), Alternatively, a *Fla*$^-$ (flagellaless, nonmotile) recipient was used in NGA without antiserum. Individual Fla$^+$ swarms were then typed for their flagellar antigens. The application of this procedure to the

present problem depends on the demonstrated linkage of *Fla* and H_1 factors (STOCKER *et al.*, 1953; 3.4, 3.7).

2.3 Serotypes were characterized by routine methods (EDWARDS and EWING 1955) of slide agglutination in 1:100 antiserum. Live cell suspensions were emulsified from colonies or used directly from penassay broth cultures. A number of the serotypes have been confirmed by tube agglutination tests in several laboratories. When the alternative phases were not both obvious on routine tests, they were ascertained after selection in NGA with antiserum against the existing phase. Most of the serums were standard reagents, i.e., sterile cresolated preparations of rabbit antiserums that had been absorbed to remove somatic and heterologous antibodies, with a tube agglutination titer of at least 1:10,000. They were used at a dilution of 1:1000 for selection in NGA.

2.4 The principal markers for this study were the naturally occurring alleles of H_1 and H_2 in the following stock cultures (STOCKER *et al.* 1953; LEDERBERG and EDWARDS 1953): *S. typhimurium*, TM2, i:1.2; *S. abony*, CDC-103, b:enx; and *S. heidelberg*, SL-28, *Fla*$^-$ r:1.2. *Gal*$^-$ (galactose-negative) mutants of *S. typhimurium* and of *S. abony* were obtained after UV-irradiation and scored on EMB galactose agar (LEDERBERG 1950). Additional *Fla*$^-$ mutants were obtained in *S. typhimurium* from UV-irradiated suspensions by plating them in NGA at a density of one to two hundred survivors per plate. The plates were inoculated three to six hours at 37°C to allow motile clones to initiate small swarms. They were then held an additional twelve to eighteen hours at 25°C at which temperature the gelatin would set and restrict further swarming. However, the clones could continue to grow to an easily visible density in situ. The *Fla*$^-$ mutants were then readily distinguished as compact spherical colonies, in contrast to the diffuse nebulae of the parental *Fla*$^+$ clones. Eight *Fla*$^-$ mutants were isolated, of which five were sufficiently stable to be tested further.

3. RESULTS AND CONCLUSIONS

3.1 *Serum selection; mixed recipients.* The leading precedent for alternative manifestation of serotypic potentialities is undoubtedly the study of *Paramecium aurelia* by SONNEBORN and his coworkers (BEALE 1954) whereby the cytoplasm was shown to be propagated in one of several inter-transformable states which determine the pattern of genic expression. If a principle of complete cytoplasmic determination were applicable to Salmonella phase variation, the phase should be inherited entirely from the recipient in transduction, in view of the disproportionate mass of the intact cell in comparison with that of the phage-borne fragment. The first experiments were therefore designed to establish whether the donor phase had any influence on the issue of transduction. Phage lysates were prepared from single colony cultures of *S. typhimurium* (i:1.2) whose purity of phase was controlled by slide agglutination tests on twenty or more colonies. All cultures used in these experiments consisted of at least ninety percent of cells in the indicated phase, and were more often entirely homogeneous over the sample of twenty subclones. For the recipient, an equal mixture of b:enx and b:**enx** cells of *S. abony* was used so that the only variable would be the phase of the donor. The phage and cells were mixed and planted on NGA agar plates containing b and enx antiserum, and the resulting swarms isolated

and characterized as follows:

		i:enx	b:1.2	Total swarms tested
a)	1:1.2 —x b:enx	19	0	19
b)	i:**1.2** —x b:enx	14	14	28

For this 2 × 2 table, $\chi^2 = 15$, and $P < .01$. Similar results were obtained in repetitions of this design, and it was concluded that the donor phase does influence the results of transduction, contrary to the cytoplasmic hypothesis.

3.2 The absence of b:1.2 types in experiment a) above might be explained by the inherent nontransducibility of $H_2^{1.2}$ from phase-1 cells in which it is not expressed, or by the persistence of its inactivity in the recipient. On the latter view, the transduction of $H_2^{1.2}$ would result in a b:1.2 recombinant, which would not swarm, however, due to its inhibition by the anti-b serum. That is, in these experiments, a new genotype will only be detected if it inherits the appropriate phase as well as antigenic potentiality. Some more direct substantiation for this assumption will be offered in the following sections.

3.3 *Motility selection: H_1 linked to Fla.* From the influence of the donor phase 3.1 it can be concluded that the control of phase is associated with the transduced fragments, i.e., that it is linked either to H_1 or H_2 or both. The following experiments were designed as further tests of the assumptions of 3.2, by studying the inheritance of phase in transductions of H_1 as an unselected marker linked to a selected *Fla*$^+$ factor.

3.4 The first of these experiments used SL 28, the naturally occurring *Fla*$^-$ mutant of *S. heidelberg*, *Fla*$^-$ r:1.2. Phase variation in this culture cannot be detected by phenotypic difference, as no flagella or flagellar antigens are produced, but can be determined after *Fla*$^+$ has been restored either by (rare) reversions or transduction from another strain. Preliminary trials of *S. abony* —x SL28 were made on fresh single colony cultures, and two of them were saved when their motile progeny were preponderantly r:1.2 and r:**1.2** respectively. Each phase was then used as a recipient with lysates from *S. abony*, b:enx and b:**enx** respectively, according to the procedure of 2.2B with results shown in table 2.

3.5 Columns 1 and 2 indicate the transductions of *Fla*$^+$ uncoupled with H_1^b and verify the stability of the two phases in the recipient. Columns 3 and 4 show the transductions in which *Fla*$^+$ was coupled with H_1^b . This was immediately discernible

TABLE 2

Transduction of H_1^b *coupled with* Fla$^+$ S. abony Fla$^+$ *b:enx* —*x* S. heidelberg Fla$^-$ *r:1.2*

Phase of transductional parents		Fla$^+$ transductional clones				
Donor	Recipient	(1) r:1.2	(2) r:**1.2**	(3) b:1.2	(4) b:**1.2**	Total tested
b:enx	r:1.2	21	0	22	0	43
b:enx	r:**1.2**	0	7	1	42	50
b:**enx**	r:1.2	11	0	30	0	41
b:**enx**	r:**1.2**	1	10	1	38	50

for **b**:1.2 types, and could be determined after the alternative phase was scored in b:**1.2** vs. r:**1.2** types. The b:**1.2** type is especially important as it verifies directly that a b phase can be transduced in an inactive form. In the experiments conducted by antiserum selections (3.1, 3.10), such recombinant clones would be suppressed.

3.6 Table 2 also shows that the recipient governs the yield of types, and the donor phase has no effect. This apparent conflict with the previous results can be resolved by recalling that in 3.1 both H_1 and H_2 transductions were enumerated, here only H_1. There is also an unexplained difference in the frequency of linked and unlinked transductions in the different combinations. It is not known whether the discrepancy is systematically related to the phases of the donor and recipient, or is simply a casual peculiarity of a single lysate. No such effect was noted in the comparable experiment of table 4.

3.7 To complete an experimental design similar to 3.4 and table 2 in the more closely studied system *S. abony* —x *S. typhimurium*, a number of *Fla*$^-$ mutants of the latter were examined for linkage to H_1 and H_2. Each *Fla*$^-$ mutant was tested x— *S. abony* **b**:enx and b:**enx**, and *Fla*$^+$ progeny examined for the presence of the b and enx antigens. Three of the eight *Fla*$^-$ mutants reverted too readily to be useful in these experiments. Of the other five, one, SW-1157 showed occasional coupling of *Fla*$^+$ with H_1^b (table 3). None showed linkage of *Fla* with H_2. The *Fla*$^-$ mutants were also tested against one another and with a number of other *Fla*$^-$ stocks and found to transduce motility to one another in all combinations, i.e., they carry *Fla*$^-$ mutations at distinctive loci (cf. STOCKER *et al.* 1953).

TABLE 3

Linkage relations of Fla$^-$ *mutants from* S. typhimurium *i:1.2*

Mutant	*Fla*$^+$ selections			
	Fla$^-$ x— *Fla*$^+$ **b**:enx tests *Fla-H*$_1$ linkage		*Fla*$^-$ x— *Fla*$^+$ b:**enx** tests *Fla-H*$_2$ linkage	
	b:1.2	i:1.2	i:**enx**	i:**1.2**
1	0	39	0	16
2	0	48	0	28
3	0	12	0	7
4	0	35	0	15
5 (SW-1157)	5	40	0	28

TABLE 4

Transduction of H_1^b *coupled with* Fla$^+$ S. abony Fla$^+$ *b:enx* —*x* S. typhimurium *SW1157* Fla$^-$ *i:1.2*

Phase of transductional parents		*Fla*$^+$ transductional clones				
Donor	Recipient	(1) i:1.2	(2) i:**1.2**	(3) **b**:1.2	(4) b:**1.2**	Total tested
b:enx	**i**:1.2	80	1	14	0	95
b:enx	i:**1.2**	3	86	0	27	116
b:**enx**	**i**:1.2	68	4	18	0	90
b:**enx**	i:**1.2**	1	90	0	20	111

3.8 In a further search for technically useful linkages, a series of twenty-six auxotrophic markers (DEMEREC, BLOMSTRAND, and DEMEREC 1955) was tested for linkage to the *Fla-H*$_1$ segment. *Fla*$^+$ selections from the various *Aux*$^-$ *Fla*$^+$ —x *Aux*$^+$ *Fla*$^-$ transductions were tested, but each of 1352 isolates was still *Aux*$^+$ (prototrophic), implying that none of these markers is linked to *Fla*.

3.9 The experiment outlined 3.4 was repeated with *S. abony* —x SW-1157, with entirely analogous results, except that the proportion of linked H_1^b-*Fla*$^+$ transductions was uniformly lower (table 4).

3.10 *Serum selection, labelled recipients.* The next experiments were a study of all four combinations of donor and recipient phase under serum selection. In order to improve the comparability of the experiments, the recipient was a mixture of phases, distinguished by a *Gal* marker. That is, from a *Gal*$^-$ mutant of *S. typhimurium*,

TABLE 5

Transductions of S. abony *and* S. typhimurium
Serum-selection; Gal-labelled recipients

i:1.2 —x b:enx; b, enx serum						b:enx — i:1.2; i, 1.2 serum					
Donor	Recipient		Transduction clones			Donor	Recipient		Transduction clones		
	Phase	Gal	Gal	i:enx	b:1.2		Phase	Gal	Gal	b:1.2	i:enx
i:1.2	b:enx	+	+	15	0	b:enx	i:1.2	+	+	10	0
	b:enx	−	−	1	0		i:1.2	−	−	0	0
i:1.2	b:enx	+	+	6	8	b:enx	i:1.2	+	+	4	16
	b:enx	−	−	2	11		i:1.2	−	−	0	34
i:1.2	b:enx	−	−	10	0	b:enx	i:1.2	−	−	16	1
	b:enx	+	+	0	0		i:1.2	+	+	0	0
i:1.2	b:enx	−	−	10	7	b:enx	i:1.2	−	−	6	19
	b:enx	+	+	1	8		i:1.2	+	+	0	29

TABLE 6

Summary of all experiments similar to table 5

Donor	Recipient	Relative incidence of transduction clones			
		Table 5		All experiments	
		d_1:r_2	r_1:d_2	d_1:r_2	r_1:d_2
d_1:d_2	r_1:r_2	51	1	127	7
d_1:d_2	r_1:r_2	1	0	3	2
d_1:d_2	r_1:r_2	26	50	108	155
d_1:d_2	r_1:r_2	3	82	3	185

colonies in either **i**:1.2 or i:**1.2** phase were picked, and the pure-phase culture mixed with the alternative phase from the *Gal*$^+$ strain. The mixed cells were then treated with phage from *S. abony* and inoculated on NGA plates with i and 1.2 serum. The resulting transduction clones (either i:**enx** or **b**:1.2) were also scored for their Gal character. An analogous experiment was also conducted with the reciprocal transduction. Previous trials had shown no indication of linkage between *Gal* and the *H* markers, so that the Gal character of a new combination told the phase of the parental recipient for each transductional clone. The results are given in detail in table 5. In table 6, the corresponding results from a number of similar experiments are pooled, d_1:d_2 signifying the generalized donors, and r_1:r_2 the recipients. The qualitative features of table 6 may be summarized as follows:

	Donor	Recipient	Manifest transductions	Probable missing classes
a.	$\mathbf{d_1}$:d_2	$\mathbf{r_1}$:r_2	$\mathbf{d_1}$:r_2	$\mathbf{r_1}$:d_2
b.	$\mathbf{d_1}$:d_2	r_1:$\mathbf{r_2}$		$\mathbf{r_1}$:d_2 d_1:$\mathbf{r_2}$
c.	d_1:$\mathbf{d_2}$	$\mathbf{r_1}$:r_2	r_1:$\mathbf{d_2}$ $\mathbf{d_1}$:r_2	
d.	d_1:$\mathbf{d_2}$	r_1:$\mathbf{r_2}$	r_1:$\mathbf{d_2}$	d_1:$\mathbf{r_2}$

3.11 In this summary, the rare entries are disregarded, and may be considered to arise from a minor admixture of phase in the parents. The r_1:$\mathbf{d_2}$ types seem also to outnumber the $\mathbf{d_1}$:r_2. This discrepancy might reflect a minor difference in the efficiency of transduction of the H_1 and H_2 loci by the lysates used, or in the recovery of the two types under the conditions of selection. As indicated earlier, the missing classes are presumed to occur but are suppressed because they retain the antigenic phase of the recipient parent, for which antiserum is present in the NGA. The excess unaltered parental cells restrict the total growth of the transduction clones at the site of inoculation so that they lack an opportunity to engender phase variants that can be detected. The occurrence of the missing d_1:$\mathbf{r_2}$ type was verified in previous experiments, 3.5, 3.9; the $\mathbf{r_1}$:d_2 is hypothetical so far, owing to the lack of any means of selecting for it (e.g., another marker linked to H_2).

3.12 *Hypothetical expectations.* A number of hypotheses may be considered for their concordance with the experimental results and are listed in table 7. The only hypothesis among those listed that agrees with the data is number 5, that the "state" of the H_2 locus (or one closely linked to it) governs the phase. It is difficult to establish whether the list is exhaustive, but the following restatement may help to expose the implicit assumptions. Since the donor phase does influence the result of transduction of the *H* factors, we assume that some element associated with the factors themselves is involved. If the phage particle carries no other relevant material besides chromosome fragments, the state of the fragment itself is in question. Of the relevant possibilities, the experimental results are clearly in favor of a decisive role of the H_2 locus. This hypothesis is amplified, and the possible coordinate role of cytoplasm and other factors is discussed in 4.1.

3.13 *Consistency of the experiments.* The earlier experiments can be considered as special cases of 3.10. 3.1 can be derived by lumping the two phases of the recipient, and is qualitatively consistent with it: compare the proportions 19: 0: 14: 14 with the corresponding 130: 9: 111: 340. Tables 2 and 3 can be correlated by considering

TABLE 7

Hypotheses of phase variation and their consequences

Hypothesis	Genotype of		Types expected from							
	$r_1:r_2$	$r_1:r_2$	$d_1:d_2$ —x $r_1:r_2$		$d_1:d_2$ —x $r_1:r_2$		$d_1:d_2$ —x $r_1:r_2$		$d_1:d_2$ —x $r_1:r_2$	
			1	2	1	2	1	2	1	2
1. Cytoplasmic state	$H_1^rH_2^r\gamma^1$	$H_1^rH_2^r\gamma^2$	$d_1:r_2$	$-r_1-$	$-r_2-$	$r_1:d_2$	$d_1:r_2$	$-r_1-$	$-r_2-$	$r_1:d_2$
2. Controlling gene	$H_1^rH_2^rG^1$	$H_1^rH_2^rG^2$	$d_1:r_2$	$-r_1-$	$-r_2-$	$r_1:d_2$	$d_1:r_2$	$-r_1-$	$-r_2-$	$r_1:d_2$
3. Complementary states of H_1 and H_2	$H_1^rH_2^r$	$H_1^iH_2^r$	$d_1:r_2$	$-r_1-$	$d_1:r_2^*$ $-r_2-$	$r_1:d_2\ddagger$ $-r_1-$	$d_1:r_2\ddagger$ $-r_2-$	$r_1:d_2^*$ $-r_1-$	$-r_2-$	$r_1:d_2$
4. State of H_1	$H_1^rH_2^r$	$H_1^iH_2^r$	$d_1:r_2$	$-r_1-$	$d_1:r_2$	$r_1:d_2$	$-r_2-$	$-r_1-$	$-r_2-$	$r_1:d_2$
5. State of H_2	$H_1^rH_2^r$	$H_1^iH_2^r$	$d_1:r_2$	$-r_1-$	$-r_2-$	$-r_1-$	$d_1:r_2$	$r_1:d_2$	$-r_2-$	$r_1:d_2$
Experimental results Table 6			$d_1:r_2$ 127	$r_1:d_2$ 7	$d_1:r_2$ 3	$r_1:d_2$ 2	$d_1:r_2$ 108	$r_1:d_2$ 155	$d_1:r_2$ 3	$r_1:d_2$ 185

The symbols $-r_1-$ in columns 2 and $-r_2-$ in columns 1 stand for $r_1:d_2$ and $d_1:r_2$ respectively. In experiments like table 6, which are done in the presence of r_1 and r_2 antiserums, these types would not be recovered.

The various hypotheses are defined by the genotypes which each assigns to the two recipient phases. The corresponding donor genotypes may be written, *ex hypothesi*, by substituting the *d* for the *r* allelic symbols. The expected types are inferred by substituting H_1^d in the indicated state for H_1^r in columns 1, and H_2^d for H_2^r in columns 2. The phenotype of each transduction is then determined from the postulated genotype.

‡* Auxiliary assumptions are required to make predictions from this hypothesis when two active* or two inactive‡ loci are combined by transduction. The table shows expectations when the choice of phase from this impasse is random. On other assumptions, the results of the two tests should be similar, unless the hypothesis is reduced to a case of No. 4 or 5 by assuming that one locus outweighs the other.

only the relative incidences of $d_1:r_2$ in table 6, and ignoring the transductions of H_g listed in the $r_1:d_2$ column; then compare 22: 1: 30: 1 and 12:0: 13: 0 with 127: 3: 108: 3, respectively, which constitute excellent agreement on the principal issue of the role of the recipient (*viz.* the H_g locus) in the activity of transduced H_l factors.

3.14 *Monophasic types.* Although diphasic behavior is more prevalent, a number of Salmonella types are monophasic, e.g., *S. typhi* d:—; *S. enteritidis* gm: —; *S. abortus-equi* —:enx. Such types are especially valuable for the production of specific serums, and have been sought after for that reason (EDWARDS and BRUNER 1946). Curiously, the natural types carrying g and related antigens are almost always monophasic, despite the fact that diphasic combinations of these factors, e.g., gp:1.2 are readily synthesized by transduction. The ecological selective factors that must be involved in this peculiarity are unknown.

3.15 Apparent monophasic behavior may be complicated by serological cross-reactions between the phases. For example, *S. cholerae-suis*, var. *kunzendorf* is recorded as —:1.5 or (c):**1.5**, as selection with anti-kunzendorf 1.5 serum is occasionally but sporadically successful for the demonstration of the c phase (EDWARDS and BRUNER 1946). However other 1.5 and 1.2 serums are more consistently effective.

The different activity of different serums might be taken as evidence that they have a specific inductive as well as a selective effect on phase variation. However, the c and 1.5 phases of this serotype are shown (LEDERBERG and EDWARDS 1953, unpublished) to have a common antigen, agglutinins for which are present in the anti-kunzendorf 1.5 serum and which also tend to suppress the c as well as the 1.5 phase. This antigen (which might be a somatic rather than a flagellar component) had been disregarded previously because it is unusually labile to formalin, commonly used in setting up H-agglutination tests.

3.16 For other monophasic types, e.g., *S. abortus-equi* (a):**enx**, this contingency probably does not apply, but the hidden phase can be demonstrated either by stringent selection for rare variations (EDWARDS and BRUNER 1939) or by transduction of the H_1^a allele to other recipients (LEDERBERG and EDWARDS 1953). This type therefore has the genotype $H_1^a \mathbf{H_2^{enx}}$ but some factor is present at these or other loci which stabilizes the existing phase, and is under current study.

3.17 *S. typhi* represents still a third variety of monophasic behavior which can be represented by the genotype H_1^d H_2-deficient, for no homology with H_2 factors of other strains can be detected by transductions in either direction, although the H_1 factor is readily transduced and substituted for. *S. enteritidis*, H_1^{gm}, and related types, and some varieties of "monophasic *S. paratyphi B*", H_1^b, appear to belong in the same category.

3.18 A fourth category is represented by another strain of "monophasic *S. paratyphi B*", CDC-137, which can be described as $\mathbf{H_1^b}$ H_2^- where "–" implies a null allele always hypostatic to H_1. In transductions to other strains, this type never transfers an effective H_2 allele. However, it readily incorporates transduced H_2 factors, e.g., $\mathbf{H_1^b}$ H_2^- x— $H_1^a \mathbf{H_2^{enx}}$ to give a typically diphasic $H_1^b H_2^{enx}$. This type may be the counterpart of *S. abortus-equi* (3.16) since an absolutely stabilized H_2 factor in the hypostatic state could never be expressed, even if transduced elsewhere.

3.19 These types are enumerated to display some of the wealth of material now open for recombinational study in the Salmonella group. They also show how monophasic exceptions can be accomodated to the pattern of phase control which was deduced from typical diphasic forms.

3.20 *Antigenic mutation.* The technique of immunoselection can also be used for the selective isolation of new mutant alleles at a single locus, once the genetic structure of diphasic types has been worked out. For some time, it has been known that some serotypes which carry b occasionally generate an "artificial phase" antigen, z_{33}, when selected in b serum. Without further analysis, z_{33} might be interpreted either as a mutation $H_1^b \rightarrow H_1^{z33}$, or a phase variant in $H_1^b H_2^{z33}$. In the course of the present study, the following changes have been obtained as isolated events during selection in the homologous serums: *S. paratyphi B*, **b**:1.2 → $\mathbf{z_{33}}$:1.2, and *S. abony*, **b**: enx → $\mathbf{z_{33}}$:enx. The derived types showed typical phase variation between the indicated phases. They were also subjected to transductions to and from **b**:1.2, **b**:enx, and **i**:1.2 in which z_{33} proved to be homologous with b and i respectively. This suggests that z_{33} corresponds to the allele H_1^{z33}, and that the change from H_1^b to H_1^{z33} is a true mutation. The z_{33} shows, however, a strong antigenic relationship to the b antigen, and further studies on the differentiation of the b and z_{33} alleles should be preceded by a more detailed serological analysis of the stocks in question.

3.21 The following reports of antigenic variations should also probably be classified as mutations of the H_1 or H_2 locus as indicated, although they have not yet been analysed by transduction tests. H_1 changes: d → j; (see KAUFFMANN 1951) lv → lw (BRUNER 1954); and a complex series gm → gp, mt → gt, mt → gms, gmq → gm, gmq → gq, gmt → mt, gmt → gt, gmt → gm, and gst → gs (BRUNER 1953). H_2 changes: 1.2 → 1.2.3, 1.2.3 → 1.2 (BRUNER and EDWARDS 1948); 1.5 → 1.10, 1.5 → 1.11 (BRUNER and EDWARDS 1947).

3.22 Although the symbols for these antigens are more complex than those for b and z_{33}, they are not fundamentally different, as the formulae primarily reflect a more detailed analysis. In general, the antigenic mutations have generated forms still related to the primitive form, and mutations of the scope, for example, of b → i have yet to be observed.

4. DISCUSSION

4.1 The preceding account has supported the conclusion that phase variation can be explained by an alternation of "states" of the H_2 locus. When this gene is in the active or epistatic state, its allele is phenotypically expressed, and the H_1 factor is functionally suppressed. Alternatively, when the H_2 gene is in the inactive or hypostatic state, the H_1 factor is expressed. While the H_1 and H_2 factors are thus functionally complementary, the H_1 factor does not vary in its *heritable* state, and its activity depends on the state of the H_2 locus. Therefore, when an inactive H_1 is transduced, from a donor in phase-2 where H_2 was epistatic, to a recipient in phase-1 wherein H_2 is hypostatic, the H_1 factor can be immediately expressed. The experiments have thus indicated that the "state" of the H_2 factor is inherited as such with the transduction of H_2 specificity, while the functional state of H_1 is not. The primacy (or stability of state) of the H_2 locus in transduction does not preclude a more integrated system during vegetative growth in which the cytoplasm or the H_1 locus may still play an important role in the perpetuation of phase. For example, epistasis by H_2 might depend on local saturation by the immediate products of its action, a steady state or feedback condition which would depend in turn on the functioning of the cytoplasm and the competitive inefficiency of a stripped H_1 locus. Unfortunately, we have almost no facts on which to build speculations on the material meaning of "state": one of the first unknowns to be pursued might well be the *external* regulation of phase. Phase variation (more accurately phase stability) evidently does not depend on the actual production of the antigenic end product, since it can be demonstrated in *Fla*$^-$ mutants which are devoid of H antigens (STOCKER *et al.* 1953; 3.4, 3.7).

4.2 The flagellar antigens are known to be proteins (STOCKER 1956) but in Salmonella they have not been studied in regard to structural features such as amino acid composition, or even their basic physicochemical properties, so there is no fundamental basis for distinguishing the phases except by their serological reactivity. However, phase-2 flagella are differentially agglutinated by acridine dyes (BERNSTEIN and LEDERBERG 1955) which speaks for some overall difference in the action of H_1 and H_2. In addition, some strains show differences in motility between the two phases (EDWARDS *et al.* 1954); whether this reflects a quantitative or qualitative difference in the flagellar proteins is a nice question. There is no precise evidence on

the regulation of phase variation by external influences, partly because of technical difficulties (cf. STOCKER 1949). There has been considerable discussion of "induced phases" in Salmonella, but no evidence that antiserum plays other than a selective role in the detection of serotypic changes has been forwarded.

4.3 Throughout this paper we have referred to *serotypes* notwithstanding the use of binomial epithets and frequent allusions elsewhere to *species* of Salmonella. Bacterial taxonomy has been dominated by the principle that any clonally stable variety whose characters can be sharply differentiated constitutes a species, a definition which is at variance with the conventions for higher forms. Transductional analyses have shown that serotypes differ in regard to well defined unit markers, but the extent to which genic differences diffuse among bacterial populations in nature is unknown.

4.4 The characterization of serotypes in this work has depended on the KAUFFMANN-WHITE scheme and on the reagents prepared to its specifications. Its authors have cautioned that the scheme is an abbreviation for diagnostic purposes, and not a complete exposition of the antigenic individuality of the serotypes. For example, the symbol "d" does not always betoken an identical antigenic constitution, and minor differences are often detectable by cross-absorption tests. The extent to which such differences are symbolized (e.g. in the distinction of enx from enz_{15} in contrast to the lumping of distinguishable 1.5 phases) depends on two possibly gratuitous factors, as well as historical accidents: the apparent titers of the residual antiserums after cross-absorption, and the urgency of more detailed differentiation as an element of classification. Neither of these considerations stands out as a necessary correlate of the importance of "minor" differences for genetic analysis. Unfortunately, the minor differences are not systematically catalogued, although they are usually mentioned in original publications of types. The sheer bulk of the present scheme has encouraged a more recent lumping of related serological phases, e.g., 1.2 with 1.2.3, rather than a finer analysis. The immediate practical consequence of these cautions is that the conventional instructions for the preparation of reagents should be followed explicitly rather than be substituted for by seemingly plausible deductions from the diagnostic scheme (KAUFFMANN 1951; EDWARDS and EWING 1955).

4.5 In the original classification of *Fla*$^-$ mutants (STOCKER *et al.* 1953), three were found to be closely linked to H_1 but were not identical. Many other *Fla*$^-$ mutants were examined and found to be nonallelic with one another and not linked to H_1. Further studies have shown that *S. heidelberg* SL28 also carries an H_1-linked *Fla*$^-$ mutation, as do several additional isolates that were not available earlier, including the laboratory mutant of *S. typhimurium* SW-1157, table 3. These *Fla*$^-$ mutants are all distinct from one another. Evidently, one cluster of *Fla*$^-$ mutants occurs in the neighborhood of the H_1 locus. Unfortunately, there is no reliable direct method of demonstrating linkage of one *Fla* factor to another, owing to their identity of phenotype. The non-H_1-linked *Fla*$^-$ mutants might therefore represent another compact cluster, or they might be widely dispersed (at least they are not closely linked to H_2 by a hypothetical analogy). It is impossible to judge whether the linkage of *Fla* factors to H_1 is fortuitous, or is somehow interrelated with their action on a common organelle (cf. DEMEREC *et al.* 1955). The appearance of a clusters of closely linked, nonrecurrent mutants with the same phenotype is reminiscent of the cluster of

Lac$_I$ and of *Gal* mutants in *Escherichia coli* (E. M. LEDERBERG 1952; MORSE *et al.* 1956). Allelism tests on the more limited scale of conventional technique in, say, Drosophila would probably have described each of these clusters as a set of multiple alleles at a single locus.

4.6 For the moment, the *H* factors appear to be simple alleles, but these markers are not so readily screened for rare recombinants, and they may yet prove to be compound in the sense of the histocompatibility factors in mice (GORER, unpubl., cited in ALLEN 1955). The persistent cross-reactivity of the original and variant in antigenic mutations such as b $\rightarrow z_{33}$ or 1.5 $\rightarrow$ 1.10 is the only hint in favor of this speculation, and the H_1 factors so far have retained their integrity in recombinational analysis. Preliminary chemical studies encourage the view that antigenic components of a complex such as enx are manifestations of different aspects of the same flagellar protein (NAKAYA *et al.* 1952).

4.7 In a system of DNA-mediated transduction (transformation) in *Hemophilus influenzae*, LEIDY, HAHN and ALEXANDER (1953) have described the separation of capsular determinants a and b in a fashion to suggest that these are nearly but not precisely allelic. In the pneumococcus, EPHRUSSI-TAYLOR (1951) had likewise demonstrated a series of closely linked "subunits" which control the quantitative aspects of capsular polysaccharide synthesis. Her "autogenic" transformations are readily interpreted as linked transductions of the factor complex, the "allogenic" transformations as transductions of a part only, i.e., as crossing over within the complex.

4.8 The state of a locus can be considered as a parameter of genic variation independent of mutational changes in specificity, which recalls D. LEWIS' (1954) account of fractional changes in self-compatibility genes in Oenothera. These mutant genes had lost only their function in the pollen, but retained their function and specificity in the style. The suppression of phenotypic effects of transposition of heterochromatin (E. B. LEWIS 1950) or of the *Ds* and *Mp* elements (MCCLINTOCK 1956; BRINK and NILAN 1952) may also be viewed as local changes of state, which although they depend on structural modifications nevertheless suggest how gene action may be regulated. These may therefore be considered as conceptual, if not mechanical, models of phase variation.

4.9 The cytoplasm has attracted considerable favor as the place of morphogenetic differentiation, largely because of an argument which has been well rephrased by EPHRUSSI (1953): "Unless development involves a rather unlikely process of orderly and directed gene mutation, the differential must have its seat in the cytoplasm". Most experiences with genic changes have involved sporadic, irreversible changes in specificity that qualify them very poorly for a role in development. However, the concept of local states may provide a more acceptable hypothetical basis for nuclear differentiation. This reconsideration of the nucleus has recently been bolstered by the caryonidal determination of mating type in Tetrahymena (NANNEY and CAUGHEY 1955), morphological diversification of chromosomal regions during the development of various tissues (see BREUER and PAVAN 1955), and especially the direct demonstration by KING and BRIGGS (1955) with nuclear transplantations from cells in later to earlier stages in frog development. The interplay of coordinate nuclear and cytoplasmic determiners has been emphasized by SONNEBORN (1954).

SUMMARY

1. The flagellar antigens of Salmonella types occur in two alternative phases, e.g., i and 1.2 in *S. typhimurium*. Transduction analysis had shown that each phase corresponds to a distinct locus, so that the genotypic formula of *S. typhimurium* would be $H_1^i\ H_2^{1,2}$. Phase variation therefore consists of the alternative manifestation of antigenic potentialities at these loci.

2. Transduction experiments were carried out with *S. typhimurium*, *S. abony* b:enx, and *S. heidelberg*, r: 1.2, with donor and recipient in various phases. The expression of the H_1 loci depended on the phase of the recipient (which retained the original H_2 locus), while the expression of a transduced H_2 locus depended on the phase of the donor cell. It is therefore concluded that antigenic phase is governed by H_2 locus, which occurs in either of two clonally stable (or nearly stable) states. The concept of "local state" presents a parameter of genic variation distinct from ordinary mutation, and may be applicable to other problems in genetics and morphogenesis.

ACKNOWLEDGMENTS

This work would not have been possible without the cumulative labors which are represented by the KAUFFMANN-WHITE scheme. We are particularly indebted to DR. C. C. SPICER for furnishing standard serums prepared by the Standards Laboratory, Central Public Health Laboratory, Public Health Laboratory Service, Colindale Avenue, London, England, and to DR. P. R. EDWARDS, of the Enteric Bacteriology Laboratories, Communicable Disease Center, Public Health Service, Chamblee, Georgia, for serums, cultures, and innumerable bits of advice and Salmonella lore.

We are also obliged to DR. M. DEMEREC for furnishing lysates of a number of auxotrophic mutants.

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TRANSDUCTIONAL HETEROGENOTES IN ESCHERICHIA COLI[1]

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Received April 12, 1956

THE transduction of the *Gal*$^+$ factor (ability to ferment galactose) to *Gal*$^-$ mutants of *Escherichia coli* K12 has been described in a previous report (MORSE, LEDERBERG, and LEDERBERG 1956). The galactose positive transduction clones were often found to be unstable and to throw off *Gal*$^-$ types about once per thousand divisions. We postulated that the transformed cells were heterogenotic (heterozygous for the transduced fragment), and that the instability was a result of segregation. This process has been studied in more detail with several non-allelic *Gal*$^-$ mutants. Since transduction genetics is a system analogous but not identical with sexual crossing, which also occurs in these strains, a distinctive terminology is a useful tool for integrating hypothesis and experiment. The following definitions are given for reference at this point. Their applications will be amplified in the experimental report.

GLOSSARY AND SYMBOLS

Genetic transduction—transfer of a genetic fragment from one cell to another.

Exogenote—a chromosome fragment; usually relates to the donor in transduction.

Endogenote—homologous part of the intact chromosome which corresponds to a given exogenote; usually relates to the recipient in transduction.

Syngenote—(cf. synkaryon) a cell whose genetic complement includes an exogenote (i.e. is hyperploid for a fragment).

Heterogenote—(cf. heterozygote) a syngenote in which the exogenote and endogenote differ in one or more markers.

Homogenote—(cf. homozygote) a syngenote in which the exogenote and endogenote carry the same marker.

Transduction clone—the entire vegetative progeny of a cell which has received an exogenote, including nonsyngenotic and syngenotic descendants.

Symbols: $/_{ex}$ Syngenotes will be given as endogenote $/_{ex}$ exogenote; —× Transduction will be symbolized as donor —× recipient.

CULTURES

The mutants that have been used in this study were accumulated in a variety of stocks, table 1. The *Gal*$^-$ mutations, as will be shown, are determined at different

[1] Paper No. 619 of the Department of Genetics. This work has been supported by grants (C-2157) from the National Cancer Institute, Public Health Service, from the National Science Foundation, and from the Research Committee, Graduate School, University of Wisconsin with funds allocated by the Wisconsin Alumni Research Foundation.

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TABLE 1

Description of principal cultures

Mutant[2]	Type culture	Method of isolation of *Gal*⁻ marker	Genotype[1]
Gal_1^-	W-750	UV, EMB lactose	$F^+ Lp^+ M^- Lac_1^- V_1^r$
Gal_2^-	W-902	UV, EMB galactose	$F^- Lp^+ T^- L^- Th^- Mal_1^-$[3]
Gal_3^-	W-892	spontaneous, EMB lactose	$F^- Lp^+ T^- L^- Th^- Lac_1^- Mal_1^-$
Gal_4^-	W-518	UV, EMB lactose	$F^+ Lp^s M^- Lac_1^- V_1^r$
Gal_5^-	W-677	UV, EMB galactose	$F^- Lp^+ T^- L^- Th^- Lac_1^- Mal_1^- Xyl^- Mtl^- Ara^- V_1^r$
Gal_6^-	W-2070	UV, EMB galactose	$F^+ Lp^s P^- G^-$
Gal_7^-	W-583	UV, EMB galactose	$F^- Lp^+ T^- L^- Th^- Lac_1^- Mal_1^- Xyl^- Mtl^- Ara^- V_1^r$
Gal_8^-	W-1210	UV, EMB lactose	$F^+ Lp^+ M^- Lac_1^- V_1^r$

[1] *F* compatibility factor; *M*, *T*, *L*, *Th*, *P*, *G*: auxotrophic markers for methionine, threonine, leucine, thiamin, proline, glycine. *Lac*, *Mal*, *Xyl*, *Mtl*, *Ara;* fermentation of lactose, maltose, D-xylose, mannitol, and L-arabinose. V_1^r: resistance to phages T_1 and T_5. (LEDERBERG *et al.* 1951; LEDERBERG, CAVALLI, and LEDERBERG 1952).

[2] Each *Gal* locus in this series is referable to a unique mutational event, no recurrences having yet been identified.

[3] Mal_1^-, maltose nonfermentation, is associated with nonadsorption of lambda as a pleiotropic effect (E. LEDERBERG 1955). This effect was formerly described as Lp_2^r.

but closely linked loci. These markers were also transferred to other experimental stocks by transduction. The symbols have been explained elsewhere (LEDERBERG and LEDERBERG 1953). As before Lp^+, Lp^r and Lp^s refer to three allelic states, respectively; lysogenic for the phage lambda, immune and sensitive. Where required, Lp^s derivatives were obtained by irradiating Lp^+ stocks with UV. Lp^s stocks were converted to Lp^+ (lysogenized) by exposing them to lambda. The infected clones must be carefully purified by serial colony isolation to insure a separation of stably lysogenic from infected sensitive subclones (LEDERBERG and LEDERBERG 1953).

METHODS

The basic technique is the selection and classification of *Gal* types on an indicator medium such as EMB galactose agar. Transduction was accomplished by mixing cells of one genotype with phage from another on agar plates as described previously (MORSE, *et al.* 1956). Two kinds of lysates were used: HFT (high frequency of transduction) from syngenotic bacteria, and LFT (low frequency of transduction) from nonsyngenotic bacteria. The HFT lysates were so active that Gal^+ transformation was readily detected by cross-brushing them with loopfuls of Gal^- bacteria (fig. 1). Conversely, Gal^- types could be obtained by growing Gal^+ together with the appropriate Gal^- HFT lysate, and streaking out the mixtures on EMB galactose agar.

Washed suspensions of compatible, auxotrophic bacteria were crossed on minimal agar (LEDERBERG, CAVALLI, and LEDERBERG 1953). Rare galactose positive recombinants were detected on a minimal indicator medium, EMS galactose agar (J. LEDERBERG 1950).

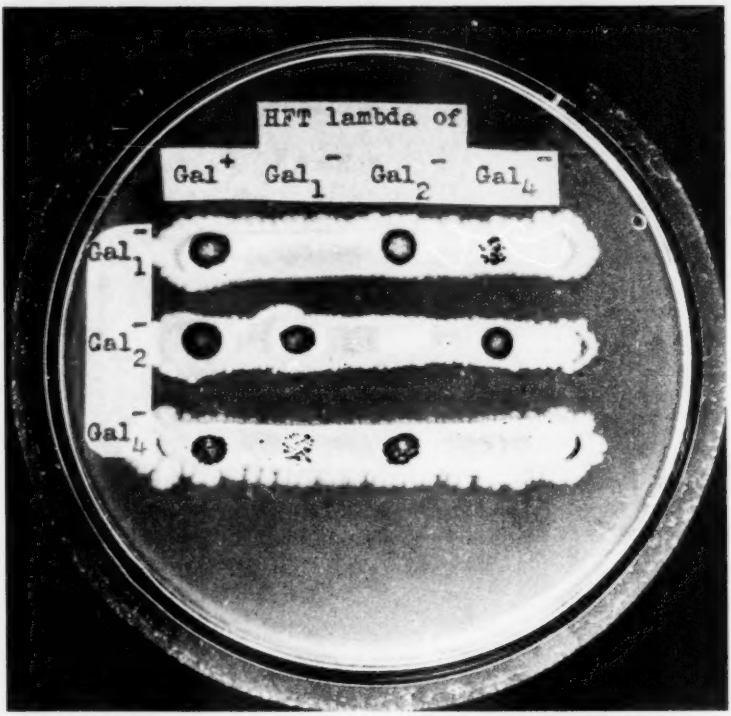

FIGURE 1.—Homology or "allelism" tests. Loopfuls of HFT lambda were spotted on cross streaks of galactose negative cultures and the plate incubated at 37 C for 48 hours. The genotypes of the cultures are given at left, the source of HFT lambda at the top. Each negative culture was transformed to a galactose positive phenotype by HFT lambda from *Gal*$^+$ and non-allelic *Gal*$^-$ cultures, but was not transformed by HFT lambda from its own Gal type. Note the lower yields of papillae in the Gal_1^-, Gal_4^- interactions. The plate shown contains a modified MacConkey's medium containing galactose instead of lactose. Routinely EMB galactose agar is used for homology tests, and with results similar to those shown, but because of its better photographic qualities (better contrast) MacConkey's was chosen for illustration.

EXPERIMENTAL RESULTS

Characteristics of Gal$^-$ mutants

The *Gal*$^-$ mutants of table 1 have the common trait of a negative (translucent, near white) reaction on EMB galactose agar. The *Gal*$^+$ type reacts by the deposition of an opaque, near purple, stain in the colony (see fig. 3, MORSE *et al.* 1956). Similarly, in fermentation tubes with galactose and bromcresol purple in peptone broth, the *Gal*$^-$ types remain negative (alkaline, no gas) for several days, while *Gal*$^+$ inocula give strong positive reactions (acid, gas) within 24 hours. Both types ferment glucose promptly. A detailed biochemical analysis of the mutants is currently under

way in KALCKAR's laboratory (KURAHASHI, unpublished; KALCKAR *et al.* 1956). Four enzymes, (1) galactokinase, (2) UDP (uridine diphospho)-transferase, (3) UDP-Gal-4-epimerase, and (4) UDP-Glu-pyrophosphatase are known to be involved in the utilization of galactose. Gal_1^- and other mutants have been found to be deficient in enzyme (2) and Gal_2^- deficient in enzyme (1).

As shown in table 1, many of the *Gal*$^-$ mutants were first recognized by their modification of lactose fermentation, and detected on EMB lactose agar as stable variants of Lac$^-$ *mutabile* stocks (E. LEDERBERG 1952). The mutants are indistinguishable on EMB galactose agar but differ slightly in their effects on lactose fermentation; Gal_1^-, Gal_4^- and Gal_7^- show a weaker Lac$^+$ phenotype on EMB lactose agar than do Gal_2^- and Gal_8^-.

The *Gal*$^-$ stocks also differ in their spontaneous revertibility to the galactose-positive phenotype, as shown by counts of papillae on confluently grown plates of EMB galactose agar. As recorded for control plates in transduction assays (tables 1, 2, 7, MORSE *et al.* 1956) Gal_4^- will usually give about 40 spontaneous papillae per plate, while Gal_1^- gives about 1 per plate of about 10^{10} cells, and the other mutants are intermediate. The rates of mutation have not been precisely measured, and the quoted figures do not distinguish mutant clones in the inocula from plate mutations. Whether the qualitative differences in revertibility are determined by the mutant loci or by modifiers has not been explicitly tested. Furthermore, many of the apparent reversions may be changes at loci other than the *Gal*$^-$ involved. Throughout these experiments, control platings have been stressed to minimize confusion from spontaneous reversals of phenotype. When these must be rigorously excluded, double mutants, e.g., Gal_1^- Gal_4^- may be used as they have not yet been found to give galactose-positive reversions.

In addition to the distinct galactose-negative mutants just summarized, a variety of weak positive phenotypes have been noted. These are typified by "Gal_5^-", which was unfortunately incorporated in an important series of multiple marker stocks of *E. coli* K-12, W-677 and W-1177 (LEDERBERG *et al.* 1951). These lines originally carried Gal_7^-, but when this was found to interfere with the expression of the Lac marker, a phenotypic reversal was selected. After UV treatment, the Gal_5^- mutant was then obtained, and was chosen for further pedigrees because it did not interfere with *Lac*, although it later proved to be a slow positive. These stocks have evidently accumulated several modifiers, and crosses involving them have given such a variety of galactose phenotypes (cf. contrasting designations by WOLLMAN 1953; CLOWES and ROWLEY 1954) that their further use, particularly where the *Gal—Lp* region is involved, should be discouraged. Gal_5 itself is not closely linked to the other *Gal* mutants and is not subject to transduction by lambda.

Differentiation of the Gal loci by crosses and by transduction

The most direct means of testing for the allelism of two *Gal*$^-$ mutants is to search for galactose positive recombinations. As a necessary control on this procedure, *Gal*$^-$ "self crosses" (e.g., Gal_1^- by Gal_1^-) were also conducted wherever possible: in these crosses no Gal$^+$ recombinants were detected (table 2). More extensive tests may well be expected to yield some galactose positives by spontaneous reversion,

TABLE 2

Self-crosses of Gal⁻ *mutants*

Cross[1]	Number of recombinants	
	Gal⁺ prototrophs[a]	Total prototrophs[b]
Hfr M⁻ × *F*⁻*T*⁻*L*⁻*Th*⁻		
Gal_1^- × Gal_1^-	0	4,200,000
Gal_2^- × Gal_2^-	0	140,000
Gal_4^- × Gal_4^-	0	800,000
Gal_6^- × Gal_6^-	0	160,000
Gal_7^- × Gal_7^-	0	120,000

[1] Approximately 10^8 cells of each parent were grown together in broth for three hours, after which time aliquots were a) plated on a minimal medium whose sole carbon source was galactose; b) diluted and plated on a minimal medium with glucose as the carbon source.

which is probably the chief limitation to the resolving power of this method. Pairwise crosses of different *Gal*⁻ gave a small fraction (about one per thousand) of *Gal*⁺ prototrophs, which indicates that they are different but closely linked loci (table 3).

The loci Gal_1, Gal_2, and Gal_4 have been most widely used, and have been testcrossed in all combinations; a few additional crosses are also listed in table 3. The loci have also been differentiated by transduction: this is shown diagrammatically in table 4. With HFT lysates, several million homology tests may be performed on a single EMB galactose agar plate (fig. 1). Therefore, HFT lysates, rather than LFT, have been used as the standard reagents of the allelism tests.

The transduction data summarized in table 4 therefore permit seven loci to be distinguished, despite occasional gaps. These distinctions are entirely consistent with those of testcrosses, insofar as these have been made. About 50 additional *Gal*⁻ mutants which can be transformed by various lysates have been isolated, but have not been completely analysed.

Segregation from syngenotes

Many of the *Gal*⁺ clones formed by the transduction, *Gal*⁺ —× *Gal*⁻, i.e., *Gal*⁺ lysate and *Gal*⁻ cells, are unstable for this trait (see also MORSE *et al.* 1956, fig. 3 and table 4). The instability is attributed to segregation because the *Gal*⁻ clones which reappear are always the same type as the original *Gal*⁻ recipient (table 5). To generalize the test as far as possible, each *Gal*⁻ segregant was picked from a heterogenotic clone which had been isolated from an independent transductional event. The segregants were typed by their reactions with various HFT reagents. Double mutants are characterized by their failure to react with either of two reagents, though they respond to *Gal*⁺ and other *Gal*⁻ lysates. Throughout this paper, the stated formula has been inferred from this test, together with any other measures that may be indicated.

In addition, the *Gal*⁺ clones obtained by Gal_1^- —× Gal_2^-, etc., are often unstable and can give more information on the segregation process. As before, each *Gal*⁻ segregant was referable to an individual transduction clone to give a composite picture of heterogenote behavior. The tests of these segregants are given in table 5.

TABLE 3

Inter-crosses of Gal$^-$ *mutants*

A. Cross[1]	Number of recombinants	
	Gal$^+$ prototrophs[a]	Total prototrophs[b]
Hfr $M^- \times F^-T^-L^-Th^-$		
$Gal_1^- \times Gal_2^-$	11	15,000
$Gal_1^- \times Gal_3^-$	3	82,000
$Gal_1^- \times Gal_4^-$	3	90,000
$Gal_1^- \times Gal_6^-$	7	102,000
$Gal_1^- \times Gal_7^-$	5	14,000
$Gal_2^- \times Gal_1^-$	152	190,000
$Gal_2^- \times Gal_4^-$	66	400,000
$Gal_2^- \times Gal_6^-$	16	93,000
$Gal_3^- \times Gal_6^-$	36	180,000
$Gal_4^- \times Gal_1^-$	36	108,000
$Gal_4^- \times Gal_2^-$	231	125,000
$Gal_4^- \times Gal_6^-$	28	174,000
$Gal_4^- \times Gal_7^-$	10	160,000
$Gal_4^- \times Gal_8^-$	83	210,000
$Gal_6^- \times Gal_1^-$	7	180,000
$Gal_6^- \times Gal_2^-$	36	33,000
$Gal_6^- \times Gal_3^-$	248	72,000
$Gal_6^- \times Gal_7^-$	13	80,000
$Gal_7^- \times Gal_1^-$	5	110,000
$Gal_7^- \times Gal_2^-$	12	19,000
$Gal_7^- \times Gal_3^-$	127	66,000
$Gal_7^- \times Gal_4^-$	1	102,000
$Gal_8^- \times Gal_6^-$	130	81,000

B. Cross[2]	Number of recombinants	
	Gal$^+$ prototrophs	Total prototrophs
$Gal_1^- \times Gal_2^-$	11	13,700
$Gal_1^- \times Gal_3^-$	2	1,600
$Gal_1^- \times Gal_4^-$	38	11,600
$Gal_1^- \times Gal_7^-$	2	7,600
$Gal_2^- \times Gal_4^-$	13	7,400
$Gal_2^- \times Gal_8^-$	3	18,400
$Gal_3^- \times Gal_8^-$	14	17,064
$Gal_4^- \times Gal_8^-$	44	10,000

[1] See footnote, table 2, for method.

[a] See footnote [1], table 2.

[b] See footnote [1], table 2.

[2] These data are pooled from a number of experiments with different mating type combinations. The crosses were conducted on EMS galactose agar, where galactose-positive and negative prototroph recombinants are scored on the same plates. These methods require further standardization before the apparent recombination fractions can be given precise *quantitative* meaning.

TABLE 4

Differentiation of the Gal⁻ *mutants by transduction*

Recipient cells	Lysates[1]							
	Gal⁺	*Gal*$_1^-$	*Gal*$_2^-$	*Gal*$_3^-$	*Gal*$_4^-$	*Gal*$_5^-$	*Gal*$_7^-$	*Gal*$_8^-$
Gal$_1^-$	+	−	+	+	+	+	+	+
Gal$_2^-$	+	+	−	+	+	+	+	
Gal$_3^-$	+	+	+	−	+	+	+	+
Gal$_4^-$	+	+	+	+	−	+	+	+
Gal$_5^-$	+	+	+	+	+	−	+	+
Gal$_7^-$	+	+	+	+	+	+	−	+
Gal$_8^-$	+	+	+	+	+	+	+	−

[1] A suspension of each type of recipient cell was cross-brushed against the indicated HFT lysates on EMB galactose agar. + indicates that galactose-positive transformations were observed, − that they were not. For *Gal*$_3^-$ and *Gal*$_8^-$, only LFT lysates were available and the reactions were recorded from platings of 0.1 ml lysate plus 0.1 ml cell suspension.

Three kinds of exogenote are considered: (1) carrying *Gal*⁺, (2) carrying non-allelic *Gal*⁻, (3) carrying two *Gal*⁻ loci, non-allelic with the endogenotic *Gal*⁻. The segregation patterns were:

Exogenote carrying	*Gal*⁻ segregant type (percent)		
	Endogenotic	Exogenotic	Recombinant types
Gal⁺	100	0	0
Non-allelic *Gal*⁻	88	11	1
Double *Gal*⁻	88	10	2

In every combination shown in table 5, the *Gal*⁻ types which are recovered correspond exactly to the types which entered into the transduction, or to recombinations of them. The order of frequency of the segregant types was endogenotic > exogenotic > recombinant. The *Gal*⁺ clone thus behaves as if the recipient cell had received a small fragment of chromosome containing the *Gal*⁺ genes (and only these since unrelated markers are not changed). Segregation from the resulting partial diploid (heterogenote) is biased, and more frequently restores the endogenotic *Gal*⁻ phenotype. Less frequently the exogenotic *Gal*⁻ is recovered, either alone or recombined as a double *Gal*⁻. To complete the segregation pattern, stable *Gal*⁺ segregants should be demonstrated. These are distinguished less readily than *Gal*⁻ and so have not been systematically enumerated but have been isolated incidentally to a number of experiments.

Genotypic formulae for syngenotes can be written *Endogenote*/$_{ex}$*Exogenote*, e.g., *Gal*$_2^-$ *Gal*$_1^+$ *Gal*$_6^+$/$_{ex}$ *Gal*$_2^+$ *Gal*$_1^-$ *Gal*$_6^-$ for the last item of table 5. By abbreviating the same symbols, the three kinds of syngenotes summarized above can be styled as ++−/$_{ex}$+++, ++−/$_{ex}$+−+, and ++−/$_{ex}$−−+. Until relevant data are available, no implications concerning gene sequence should be read into the formulae.

The data of table 5 show no obvious difference between reciprocal transductions;

TABLE 5

Segregation from the syngenotes

Syngenote; Recipient Gal_y^- Donor Gal_z^- or Gal^+		Number of segregants observed with:			
		Gal_y^-	Gal_z^- or Gal^+	$Gal_x\ Gal_y^-$	Total
endogenote/ex exogenote		*endogenotic*	*exogenotic*	*crossover*	
$Gal_1^- Lp^s$	Gal^+	9	0	0	9
Lp^+	Gal^+	33	0	0	33
$Gal_2^- Lp^s$	Gal^+	16	0	0	16
Lp^+	Gal^+	20	0	0	20
$Gal_4^- Lp^s$	Gal^+	31	0	0	31
Lp^+	Gal^+	20	0	0	20
Lp^r	Gal^+	29	0	0	29
$Gal_8^- Lp^s$	Gal^+	29	0	0	29
Lp^+	Gal^+	15	0	0	15
		201 (100%)			201
$Gal_1^- Lp^s$	Gal_2^-	1	0	0	1
	Gal_8^-	6	1	0	7
Lp^+	Gal_2^-	36	6	0	42
	Gal_8^-	18	3	0	21
$Gal_2^- Lp^+$	Gal_1^-	14	3	2	19
	Gal_4^-	9	7	0	16
$Gal_4^- Lp^s$	Gal_2^-	18	3	0	21
	Gal_8^-	17	2	0	19
Lp^+	Gal_2^-	16	3	0	19
Lp^r	Gal_2^-	15	3	0	18
$Gal_8^- Lp^s$	Gal_1^-	40	1	0	41
	Gal_4^-	42	1	1	44
Lp^+	Gal_1^-	19	2	0	21
	Gal_4^-	22	1	0	23
		273 (87.5%)	36 (11.5%)	3 (0.9%)	312
$Gal_8^- Lp^{+1}$	$Gal_1^- Gal_4^-$	135	14	3	152
	$Gal_1^- Gal_7^-$	29	1	0	30
$Gal_2^- Lp^+$	$Gal_6^- Gal_7^-$	18	2	1	21
	$Gal_1^- Gal_6^-$	12	4	0	16
		194 (88.5%)	21 (9.6%)	4 (1.8%)	219

[1] In these transductions, involving three factors, Gal_x^- refers to both mutant loci taken together; the crossovers are other combinations.

nor is there any effect of the *Lp* allele of the recipient on the segregation pattern of the resulting syngenotes. As noted previously (MORSE *et al.* 1956) all transduction clones derived from Lp^+ recipients were lysogenic; from Lp^s recipients, Lp^+ or Lp^r; and from Lp^r recipients, usually Lp^r. The segregational behavior of *Lp* alleles is still under study and will be taken up elsewhere.

The tests for *Gal* type were based on the pattern of transduction by lysates from known Gal^- cultures. As additional checks on the transductional test for *Gal* type

TABLE 6

Examination of segregants from heterogenotic clones

Heterogenote		Classification[1] of segregant by:					
		Transduction *Gal* type	Lysate activity *Gal* type	Testcross to:			
				Endogenotic *Gal*$^-$		Exogenotic *Gal*$^-$	
				Gal$^+$	total	*Gal*$^+$	total
$Gal_2^- / _{ex}\ Gal_2^+$	1.	Gal_2^-	Gal_2^-	0	4070		
	2.	Gal_2^-	Gal_2^-	0	5384		
	3.	Gal_2^-	Gal_2^-	0	2072		
	4.	Gal_2^-	Gal_2^-	0	6988		
$Gal_4^- / _{ex}\ Gal_4^+$	1.	Gal_4^-	Gal_4^-	0	896		
	2.	Gal_4^-	Gal_4^-	0	918		
	3.	Gal_4^-	Gal_4^-	0	1134		
	4.	Gal_4^-	Gal_4^-	0	863		
$Gal_4^- Gal_2^+ / _{ex}\ Gal_4^+ Gal_2^-$	1.	Gal_4^-	Gal_4^-	0	2786	3	3183
	2.	Gal_4^-	Gal_4^-	0	2675	2	3471
	3.	Gal_4^-	Gal_4^-	0	3485	23	5342
	4.	Gal_4^-	Gal_4^-	0	5952	1	1665
	5.	Gal_4^-	Gal_4^-	0	5000	1	891
	6.	Gal_2^-	Gal_2^-	7	3102	0	1988
	7.	Gal_2^-	Gal_2^-	10	4364	0	1187
$Gal_4^- Gal_2^+ / _{ex}\ Gal_4^+ Gal_2^-$	1.	Gal_4^-	Gal_4^-	0	16104	0	1389
	2.	Gal_4^-	Gal_4^-	0	5730	1	164
	3.	Gal_4^-	Gal_4^-	0	3358	0	202
	4.	Gal_4^-	Gal_4^-	0	12848	1	171
	5.	Gal_2^-	Gal_2^-	1	11200	0	827
	6.	Gal_2^-	Gal_2^-	6	10608	0	718
	7.	Gal_2^-	Gal_2^-	3	5000	0	409

[1] Classifications: (1) by transduction, exposure to lysates of known *Gal*$^-$; (2) lysate activity, lysate of the segregant on known *Gal*$^-$; (3) testcrossing with known *Gal*$^-$ cultures, the figures given are *Gal*$^+$ prototrophic recombinants and total prototrophic recombinants, scored as per table 3B.

a number of segregants were tested further by two methods: (1) by making lysates of them and plating the lysate with known *Gal*$^-$ cultures, (2) test crossing against known *Gal*$^-$ cultures. These further checks on the classification gave perfect agreement with each other and with the transductional test. Cultures of 79 segregants were checked by transduction to known *Gal*$^-$, and 26 segregants were testcrossed. The scope of the analysis is indicated by the data in table 6, in which data on segregants tested by both methods are recorded.

The sequence of events in segregation from a single heterogenote could, in principle, be studied in cell pedigrees, but the rate of segregation, about 10^{-3} per division, would make this too laborious an enterprise at present. A number of individual heterogenotes have, however, been studied intensively by plating methods. For example, W2869, $Gal_4^-\ Gal_2^+ / _{ex} Gal_4^+\ Gal_2^-$ was replated after purification, and no more than one *Gal*$^-$ segregant tested per segregating *Gal*$^+$ colony. This insures the uniqueness of each

segregant. From a total of 112 colonies, the segregant types were 85 Gal_4^-; 26 Gal_2^- and 1 Gal_2^- Gal_4^-, which are endogenetic, exogenetic and recombinant, respectively.

Syngenotes are distinguished from haploids (nonsyngenotes) primarily by their genetic complexity, which is revealed by segregation. A second distinction, with technical import for further analysis, is their behavior as transductional donors. As noted previously (MORSE *et al.* 1956) the lysates from heterogenotic cultures show a very high frequency of transduction. Every Lp^+ heterogenote which has been tested has given a lysate showing HFT behavior, i.e., not less than one transduction per hundred plaque-forming particles, and often approaching one per one. Clones which give HFT lysates will be referred to as HFT^+. As will be noted later, HFT^+ behavior has served in turn as an auxiliary criterion for homogenotes, in which segregation is less readily observed.

HFT lysates from a number of double heterogenotes, e.g., $Gal_1^-Gal_2^+/_{ex}Gal_1^+$ Gal_2^-, have been assayed on various Gal^- types, with the results depicted in table 7. The exogenote is preferentially included in the phage which matures in the syngenotes. In view of the incidence of non-syngenotic segregants, which are known to generate much larger bursts of LFT phage than syngenotes, the true efficiency of phage from heterogenotes is systematically underestimated. The estimation of preferential inclusion of exogenotes is also complicated by the incidence of syngenotic crossovers, as described in the following section.

Homogenotic segregants

Three *a priori* possibilities for the constitution of Gal^- segregants may be considered (the first two of which have been realized): (1) reduced haploids; (2) unreduced syngenotes, which have become homogenotic for one or more Gal^- loci; (3) diploids homozygous for one or more Gal^- loci (these might arise by secondary nondisjunction). If segregants occur whose genotypes consist only of exogenotes, we assume they would be inviable. Each of the events might or might not be preceded by crossing over.

Two methods are available for distinguishing homogenotic ($Gal^-/_{ex}Gal^-$) from haploid, nonsyngenotic ($Gal^-/$) segregants. Reverse mutation from Gal^- to Gal^+ would convert a homogenote to a segregating heterogenote, $Gal^-/_{ex}Gal^-$ to $Gal^+/_{ex}Gal^-$ or $Gal^-/_{ex}Gal^+$. A haploid Gal^- would revert to a haploid Gal^+, which would not segregate for galactose fermentation; this supposition is supported without exception by tests of at least 100 reversions from type Gal^- cultures, usually obtained as papillae from control platings in transduction assays (see, e.g., tables 1, 2, 7, MORSE *et al.* 1956).

In addition, by analogy with heterogenotes, homogenotic cultures should be HFT^+. Haploid segregants like all of the type haploid stocks which have been tested should be LFT^+. Altogether, 77 segregants from 18 different heterogenotes were screened initially by the reversion test. For each test from one to ten independent reversions was selected from each Gal^- segregant clone. Two of the 77 (2.6%) gave segregating reversion clones and are tentatively considered to be homogenotic. Both segregants happen to come from W2869 ($Gal_4^-Gal_2^+/_{ex}Gal_4^+Gal_2^-$) and are themselves $Gal_2^-/_{ex}Gal_2^-$ homogenotes. From one of these clones, both of two reversions were unstable; from

TABLE 7

Lysates of heterogenotes

Heterogenote		Assay of lysate of heterogenote for:			Ratio exo:endo
Endogenote	Exogenote	Phage[1]	Exogenote Gal^{+}[2]	Endogenote Gal^{+}[3]	
Gal_1^-	Gal_2^-	7200	120	1	120
Gal_1^-	Gal_3^-	1	1.8	0.06	30
Gal_3^-	Gal_1^-	620	150	43	3.5
Gal_2^-	Gal_4^-	730	25	0.28	89
Gal_3^-	Gal_4^-	21	14	1.2	11

[1] Plaques on Lp^s culture.
[2] Papillae on Lp^+ endogenotic Gal^- culture.
[3] Papillae on Lp^+ exogenotic Gal^- culture.

the other, one reversion was unstable, the other stable. The last observation may be attributed to a suppressor mutation in another region, further segregation in the homogenotic clone, or to the lesser frequency of Gal$^-$ (exogenotic) segregants from the potential $Gal^+/_{ex}Gal^-$ type of reversion. Consequently, the total estimation of the incidence of homogenotic segregants is probably low, especially for segregants of which only one or a few reversions have been examined.

Both of the inferred *Gal*$^-$ homogenotic segregants were tested and found to be HFT$^+$, a result which bolsters the confidence that may be placed in this more convenient test. To simplify the test, a suspension of *Gal*$^-$ cells was held in a loop under a UV lamp, then spotted on a plate previously spread with indicator *Gal*$^-$ cells. Alternatively, such a plate may be irradiated after it has been spotted. Low doses of UV are used, which leave enough bacterial survivors, but release sufficient phage to distinguish HFT$^+$ from LFT$^+$. To classify its *Gal* type, the segregant is tested on indicators which correspond to the components of its parental heterogenote. For example, lysates of *Gal*$^-$ segregants from W-2869 and the heterogenotes listed in table 7 were tested on Gal_2^- and Gal_4^- indicators.

For a study of the incidence of homogenotic segregants, six independent heterogenotes were isolated from $Gal_4^- \times Gal_2^-$. Individual segregants of each heterogenote were tested for HFT$^+$ as shown in table 8. Altogether, of 104 segregants tested, 20 were homogenotic. However, the heterogenotes were quite disparate: one of them accounts for 16 homogenotes, and these were all of the exogenotic type.

TABLE 8

Homogenotic segregants (detected as HFT$^+$)

Number of homogenotes	$Gal_2^+ Gal_4^-/_{ex} Gal_2^+ Gal_4^-$ heterogenotes					
	293-1	293-2	293-3	293-11	293-12	293-13
$Gal_2^-/_{ex} Gal_2^-$	1	2	0	1	0	0
$Gal_4^-/_{ex} Gal_4^-$	0	0	0	0	16	0
Total tested	11	11	11	11	49	11

TABLE 9

Observations on homogenotic clones

Homogenote				LFT+ segregant	
Phenotype	Derived from		Galactose-positive reversions segregating/total	Phenotype	Galactose-positive reversions segregating/total
Gal_1^-	$1^-2^+ / _{ex}\ 1^+2^-$		4/5	Gal_1^-	0/8
Gal_2^-	$2^-4^+ / _{ex}\ 2^+4^-$	(1)	4/4	Gal_2^-	—*
		(2)	2/3	Gal_2^-	—
		(3)	2/3	Gal_2^-	—
		(4)	3/4	Gal_2^-	—
		(5)	4/6	Gal_2^-	0/5
Gal_2^-	$1^-2^+ / _{ex}\ 1^+2^-$	(1)	12/12	$Gal_1^-Gal_2^-$	none obtained
		(2)	12/12	Gal_2^-	0/12
		(3)	12/12	$Gal_1^-Gal_2^-$	none obtained
Gal_2^-	$2^+4^- / _{ex}\ 2^-4^+$	(1)	10/18	Gal_2^-	—
		(2)	2/2	—	—
		(3)	1/2	Gal_2^-	—
Gal_4^-	$2^-4^+ / _{ex}\ 2^+4^-$	(1)	1/6	—	—
		(2)	1/6	—	—
		(3)	2/4	—	—
		(4)	1/1	—	—
		(5)	3/4	—	—
Gal_6^-	$2^-6^+ / _{ex}\ 2^+6^-$		2/2	Gal_6^-	0/3
Gal_7^-	$2^+7^- / _{ex}\ 2^-7^+$		8/14	Gal_7^-	0/7

* — signifies not examined.

Further segregation from the HFT+ homogenotes cannot be detected by instability of the *Gal* phenotype. It is, however, reflected (1) in the segregation of Gal^+/Gal^- heterogenotes which result from reversion of one Gal^- allele, (2) in the spontaneous occurrence of LFT+ derivatives, whose Gal^+ reversions do not segregate (table 9).

The HFT lysates from homogenotes may be exploited as pure reagents for Gal typing. The homogenotes for this purpose have been obtained from two-locus heterogenotes, in which the desired Gal^- factor was either endo- or exogenotic (table 9). At the present time, HFT lysates have been obtained for Gal_1, Gal_2, Gal_4, Gal_6, and Gal_7 but not for Gal_3 or Gal_8. In the search for the appropriate homogenotes, 385 segregants were tested, and 24 homogenotes were found. However, for the immediate purpose, the segregants had been tested on only one indicator, and some homogenotes might have been missed.

The homogenotes so far considered are not necessarily related to crossing over between the *Gal* markers. For example, the first line of table 8 shows $Gal_1^-Gal_2^+ / _{ex}Gal_1^+Gal_2^-$ giving $Gal_1^-Gal_2^+ / _{ex}Gal_1^-Gal_2^+$, while the seventh line shows the alternative $Gal_1^+Gal_2^- / _{ex}Gal_1^+Gal_2^-$ homogenote. This formulation is based on the Gal typing of the LFT+ segregants. However, segregants of the form $Gal_1^-Gal_2^- / _{ex}Gal_1^+Gal_2^-$ have also been identified. They give the same typing reactions (i.e., Gal_2^-) to HFT reagents as $Gal_1^+Gal_2^- / _{ex}Gal_1^+Gal_2^-$, but they continue to segregate to give $Gal_1^-Gal_2^-$ types. From these results we would infer that segregation may coincide with crossing

over, whether syngenotic or non-syngenotic types result. Obviously, the crossover syngenotes could result only from a system of crossing over involving more than two strands.

On a number of structural hypotheses, crossing over and segregation in a syngenote might lead to diploidization and homozygosity for markers not initially involved in transduction. This can be tested by the segregation of reverse mutations of the indicated marker as has already been described for *Gal* homogenotes, and previously used for the discrimination of hemi- from homozygous diploids (LEDERBERG *et al.* 1951). The markers: Lac_1, Mal_1, Xyl_2, Ara_2, have been used for this purpose. The tests, while not very extensive, indicate that homozygosity for these markers is not correlated with segregation for *Gal* from heterogenotes.

Segregation from heterogenotes showing position effect

Under the usual assay conditions, some transductional combinations of one Gal^- with another give galactose positive clones with the same frequency and rapidity as transductions from Gal^+ to Gal^-. $Gal_1^- \times Gal_2^-$, $Gal_2^- \times Gal_4^-$, and their reciprocals are included in this category; $Gal^+ \times Gal_1^- Gal_4^-$ is equally prompt. On the other hand, $Gal_1^- \times Gal_4^-$ and $Gal_4^- \times Gal_1^-$ give lower yields and a delayed development of positive clones (fig. 1). As we shall see this discrepancy can be explained by the galactose-negative phenotype of the $1^+4^-/_{cz}1^-4^+$ and $1^-4^+/_{cz}1^+4^-$ heterogenotes, which can be isolated from appropriate mixtures of cells and HFT lysate on EMB galactose agar. The heterogenotes can be distinguished from the nonsyngenotic Gal^- recipients by the frequent development of positive papillae and sectors after two to three days incubation. The heterogenotes $1^-4^-/_{cz}1^+4^+$ and $1^+4^+/_{cz}1^-4^-$ have also been prepared and found to be galactose positive. The various combinations and phenotypes can thus be summarized:

trans	trans	cis	cis
$+\ -/_{cz}-\ +$	$-\ +/_{cz}+\ -$	$-\ -/_{cz}+\ +$	$+\ +/_{cz}-\ -$
negative	negative	positive	positive

The pattern is diagnostic of a cis-trans position effect.

The constitutions of these heterogenotes are primarily inferred from their immediate parentage, e.g. $1^+4^-/_{cz}1^-4^+$ is considered to be the typical result of $1^-4^+ \times 1^+4^-$. For the present we shall be concerned principally with their segregational behavior, which is consistent with their inferred constitution and follows the same lines as segregation in other heterogenotes (table 10). The interactions of these and other *Gal* loci in heterogenotes and heterozygotes are being studied in more detail for presentation elsewhere.

The galactose-positive cis heterogenote $1^-4^-/_{cz}1^+4^+$ was obtained from $Gal^+ \times Gal_1^- Gal_4^-$. (The latter was isolated as a segregant from a heterogenote $1^+4^-/_{cz}1^-4^+$, table 10). The opposite arrangement, $1^+4^+/_{cz}1^-4^-$ could not be directly selected, but was obtained as the galactose positive heterogenote $1^+4^+8^-/_{cz}1^-4^-8^+$, by the transduction $Gal_1^- Gal_4^- \times Gal_8^-$. The trans-heterogenotes were isolated as already de-

TABLE 10

Segregation from heterogenotic clones showing position effect

A. The *cis*-position, galactose positive clones

Heterogenote		Segregants with *Gal* genotype			
Endogenote	Exogenote	Gal_3^-	$Gal_1^- Gal_4^-$	Gal_1^-	Total
I^-4^-	I^+4^+	0	24	0	24
$8^- I^+4^+$	$8^+ I^-4^-$	135	14	3	152

B. The *trans*-position, galactose negative clones

Heterogenote		Segregants with *Gal* genotype			
Endogenote	Exogenote	Gal_1^-	Gal_4^-	$Gal_1^- Gal_4^-$	Total
I^-4^+	I^+4^-	21	3	0	24
I^+4^-	I^-4^+	4	14	0	18

C. The galactose positive clones (crossover syngenotes) occurring in *trans*-position clones[1]

Crossover syngenotes in *trans*-position heterogenotes with:		Segregants with *Gal* genotype				
Endogenote	Exogenote	Gal^+[2]	Gal_1^-	Gal_4^-	$Gal_1^- Gal_4^-$	Total
I^-4^+	I^+4^-	9	2	3	0	14
I^+4^-	I^-4^+	12	4	4	3	23

[1] After 24–36 hours the *trans*-position galactose negative colonies papillate and show, after restreaking, galactose positive clones, which may or may not segregate galactose negatives. Each segregant listed was obtained from a separate rearrangement to give the galactose positive phenotype. In addition to the Gal^- types listed as obtained from the rearrangements, some papillating galactose negatives were also obtained. The latter are presumed to be rearrangements from $+ +/_{ex} - -$ to $+ -/_{ex} - +$ and $- +/_{ex} + -$.

[2] Clones listed under this column failed to segregate in two streakings. They may have a $+ +$ endogenote or they may be haploid $+ +$.

scribed. Gal^- segregants were detected by their failure to papillate. Galactose-positive segregants were ignored for this tabulation, but are considered below. The segregation patterns for these heterogenotes are given in table 5.

In addition, table 10 presents the segregational patterns of galactose-positive derivatives, isolated as papillae or sectors from trans-heterogenotic colonies. Some of the galactose-positives failed to segregate in the routine test, and are assumed to carry I^+4^+ crossovers. The segregants might include non-syngenotic haploids, and syngenotes with a $+ +$ endogenote whose segregation would not be readily detected. Segregating galactose-positive clones included types inferred from single segregants $+ -/_{ex} + +$, $- +/_{ex} + +$, and $- -/_{ex} + +$, as listed in table 10. The "galactose-negative segregants" also included colonies which later papillated, and thus resembled the original trans-heterogenotes. These are readily interpreted as $+ -/- +$ crossover syngenotes, resulting either from $- -/_{ex} + +$ or $+ +/_{ex} - -$ galactose-positive heterogenotes.

DISCUSSION

During the past 30 years, non-sexual processes of genetic recombination have been been discovered in a variety of bacterial species: pneumococcus (GRIFFITH 1928; HOTCHKISS 1955; EPHRUSSI-TAYLOR 1955); Hemophilus (ALEXANDER and LEIDY 1951); Neisseria (ALEXANDER and REDMAN 1953); Salmonella spp. (ZINDER and LEDERBERG 1952; BARON 1955; ISEKI 1954; EDWARDS 1955); *Escherichia coli* (LENNOX 1955; JACOB 1955; MORSE *et al.* 1956). The common feature of the examples that have just been cited is the transmission of a small hereditary fragment by a cell-free preparation. The same examples differ in their details, some being mediated by free desoxyribonucleic acid (DNA) and others entailing phage particles as a vector. To emphasize a unitary genetic interpretation of these examples, and to distinguish them from sexual processes, the term transduction (J. LEDERBERG 1952) was proposed for the transfer of genetic fragments. Some workers have since used "transduction" more narrowly, to distinguish phage-mediated from DNA-mediated "transformation", but we are more concerned with the unified interpretation of these phenomena than with the rigidification of the nomenclature.

The principal features of the *E. coli*-lambda system of transduction may be summarized as follows:

1. The transduced markers. A large number of galactose-nonfermenting (*Gal*$^-$) mutants have been obtained in *E. coli* K-12. Although these mutants are phenotypically similar, each of the first seven occurrences to be analysed has been related to a distinct locus, separable from the others by recombination tests. These tests have included sexual crosses, as well as reciprocal transductions, with concordant results in each case. The various *Gal*$^-$ loci are closely linked to one another and to the Lp locus, the determinant of lambda-lysogenicity.

2. Transduction by lambda. Ordinary cultures of lysogenic (*Lp*$^+$) bacteria produce lambda sporadically and to a low titer. When these cells are exposed to ultraviolet light (UV) a large proportion of them are induced to yield free lambda, with concomitant lysis of the bacteria (WEIGLE and DELBRÜCK 1951). Occasional particles of "induced lambda" from *Lp*$^+$ cells contain the linked *Gal* genes, in addition to the lambda prophage itself. This transduction of *Gal* markers is revealed by the transformation of *Gal*$^-$ cells to galactose-positive phenotypes. The specificity of this transformation rules out general mutagenic effects: lysates of *Gal*$^-$ cultures do not transform homologous types, but will transform each of the heterologous mutants. No markers other than *Gal* have been found to be transduced by lambda.

That the lambda particle itself is the active component of the lysate is indicated by the correlation of lysogenizing with transducing activity. Lysogenic, immune, and sensitive cultures (*Lp*$^+$, *Lp*^{r} and *Lp*^{s}, respectively) are all effective recipients of transduction by lambda.

Lambda can also be produced by its lytic growth on *Lp*^{s} bacteria. In contrast to the "induced lambda" from *Lp*$^+$ bacteria, this "lytic lambda" is not demonstrably effective in transduction.

3. Segregation in transformed clones. The transformed clones resulting from transduction fall into two categories. About a third proved to be stable, positive types and behaved like the wild types in further culture. About two thirds of the trans-

formed clones are unstable and more attention has been given to these types. Even after repeated purification the "segregating" clones throw off galactose-negative progeny at a rate of about one per 1,000 cell divisions. This rate is, however, variable from clone to clone and some so-called stable clones may be misclassified. The study of the unstable positive clones obtained from the combination of heterologous lysates and recipient cells justifies the following conclusions: (a) The unstable positive clones are heterozygous for the recipient and donor Gal markers but not for any other marker that has been tested. The term heterogenote is proposed for this type of partial diploid, which carries a transduced fragment or exogenote in addition to the basic genome. This structural interpretation is indicated by the reappearance among the segregants of the two specific *Gal*$^-$ types which had entered into the transduction. In addition to the parental combinations, recombinations are also observed among the segregants. The recombinations include both the double *Gal*$^-$ and the double *Gal*$^+$, or wild type. (b) Most of the segregants appear to be haploid but about 6% are homogenotic for one of the *Gal* mutant markers. A consideration of these derived homogenotes shows that recombination can take place at a more than two-strand stage. (c) The most frequent type of segregant corresponds to the marker originally carried by the transductional recipient. The next most frequent type of segregant carries the *Gal* marker of the transductional donor and the least frequent type is a recombinant with respect to the two sets of markers.

4. Transductional properties of heterogenotic clones. As previously indicated, induced lambda from haploid $L\rho^+$ cultures has a low frequency of transduction, about 10^{-6} per phage particle. Induced lambda from heterogenotic clones has a much higher frequency of transduction approaching one per phage particle. Differential assays of these lysates indicate that the exogenote is preferentially included in the phage.

5. Phenotypic properties of heterogenotic clones; position effect. The occurrence of galactose-positive heterogenotes from the transductions of *Gal*$^+$ to each of the *Gal*$^-$ markers indicates that the *Gal*$^+$ allele is dominant. In addition, all of the *cis* double heterogenotes of the form $- -/_{ex} + +$ have been galactose-positive, as have been many but not all of the *trans* $+ -/_{ex} - +$ combinations. Among the types summarized in this paper, the particular combination $1^-4^+/_{ex}1^+4^-$ has proved to be galactose-negative, as has the reciprocal $1^+4^-/_{ex}1^-4^+$. The contrast between the phenotype of the two arrangements in this case thus indicates a *cis-trans* position effect. The structures of the *cis* and *trans* heterogenotes have been inferred both from their mode of formation and from their segregational pattern. The Gal_1, Gal_2, and Gal_4 markers have been most thoroughly studied from this point of view: in this group Gal_1 and Gal_4 show the positional interaction while none of the other combinations do so. Further studies involving the other *Gal*$^-$ markers are in progress.

The first stage of transduction is the disruption of the donor genotype. In the DNA-transductions, this is accomplished when the cells are dissolved in bile salts; in phage-mediated transductions, it is an incident of phage growth. In Salmonella and the pneumococcus, any marker is readily transducible; the inclusion of exogenotes in Salmonella phage particles may be purely adventitious. In the lambda system, however, two restrictions are noted; 1) the phage, to be effective, must be prepared by the "induction" of a lysogenic bacterium ($L\rho^+$), and 2) the only factors that can be

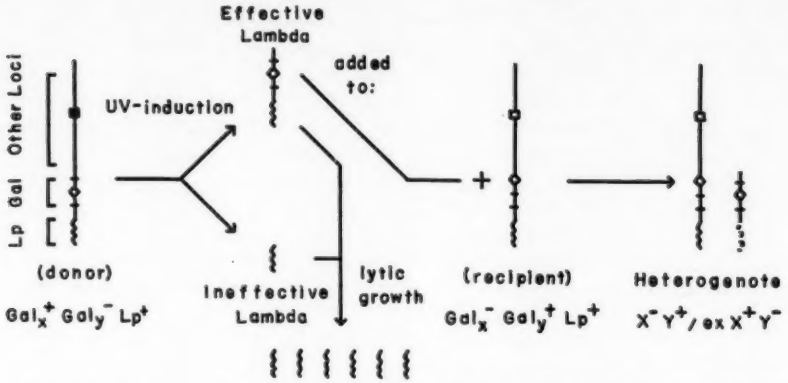

FIGURE 2.—Diagrammatic representation of the lambda-*E. coli* transduction system. The fate of the prophage (Lp) segment has not been worked out: this is indicated by the broken outline in the diagram of the heterogenote and by the omission of this segment from figures 3 and 4.

transduced are the *Gal* markers. Since it is precisely these markers which are linked to *Lp* (in crosses, LEDERBERG and LEDERBERG 1953; in transduction, LENNOX 1955 and JACOB 1955), we conclude that the effective exogenotes have a special relationship to lambda prophage. The simplest view of this relationship is diagrammed in figure 2, that the *Gal* markers and the prophage (*Lp*$^+$) are adjacent segments of the bacterial chromosome, and that an effective particle is one in which induction has accidentally released a fragment which includes the *Gal* region, as well as the unique *Lp* segment. From this point of view, lysogenization, or the transfer of the prophage is itself a form of transduction, in which the behavior of *Lp* (prophage) is analogous to that of *Gal*. The unique features of the *Lp* segment which relate it to virus are the capacity for autonomous growth of *Lp*$^+$ in the lytic cycle, and in "inducibility", i.e., the formation of specific coats in the maturation of phage when *Lp*$^+$ bacteria are exposed to UV.

The envelopment of the *Gal-Lp* segment in the coat of a mature phage particle protects this material during its extracellular existence, and provides the means for its reentry into a new bacterium (assuming an analogy of lambda with T_2, HERSHEY and CHASE 1952). How these ends are accomplished with free DNA is known only on a complex, empirical basis.

At this point of our narrative, the exogenote has penetrated the recipient bacterium. This bacterium may be lysogenic, immune, or sensitive. Undoubtedly a fraction of transduction clones that would otherwise be detected from sensitive recipients are lost by lysis; how it is determined whether an infected bacterium will be lysed, lysogenized, or otherwise protected, is unknown.

The criterion for effectiveness in transduction is the formation of a galactose-positive clone, and therefore for the functional and reproductive integrity of the *Gal* segment. Ineffective particles may have exogenotes that were defectively prepared, or being intact, are improperly established in the recipient cell. The high activity of

HFT lysates shows that establishment is not a general limiting factor; the LFT lysates may be assumed to contain a heterogeneous population of particles, of which only a few contain an effective exogenote. The HFT quality of the lysates from syngenotes may be explained on the basis of selection of effective exogenotes, which had been screened by a prior selection for the ability to form a persistent galactose-positive heterogenote. The induction of a syngenote evidently preserves the integrity of the exogenote, as shown by its preferential inclusion in the phage yield, and perhaps by the reduced yield of phage from such a cell.

Two types of transformed clones have already been noted, stable and segregating. The stable clones evidently result from an early exchange of the exogenote with the recipient, leading to a reduced haploid product. The mechanism of this exchange cannot be readily studied (LEDERBERG 1955), but may be compared to the crossing over and reduction observed with persistent heterogenotes. However, the incidence of primary stable transformed clones (about $\frac{1}{3}$ of the total) is much higher than would be expected from the known rate of segregation in heterogenotic clones (10^{-3} per bacterial division, MORSE *et al.* 1956). It has also been observed that stable transduction clones are much less frequent in HFT transductions. The discrepancy might be explained either by postulating that the initial heterogenote is inherently unstable, or that LFT lysates include a moiety of particles that are capable of effective exchange, but not of initiating a persistent heterogenote. No data are yet available on the distribution of fragment sizes; the exogenotes so far studied (in HFT lysates from syngenotes) appear to encompass all the *Gal* markers.

More information about the mechanism of exchange can be obtained from the study of persistent heterogenotes. The three basic modes of division are diagrammed in figure 3. Most often, the heterogenote is propagated as such (*mitosis*); it may be *reduced* to a haploid, with or without concomitant crossing over; it may undergo internal recombination or *automixis*, i.e., engender a new syngenote of different constitution, for which one or more crossovers are implied. The types of automictic syngenotes already imply that crossing over occurs at a stage at which either the endogenote or the exogenote (presumably both) is already duplicated, i.e., at a "four-strand" stage. Proof that crossing over in a specific instance involves all four strands would entail, for example, the identification of a $+\ +/_{ex}+\ +$ homogenote from a $+\ -/_{ex}-\ +$ heterogenote. Suspected occurrences of such types have not been completely analyzed (table 10).

Without more explicit information on centromere relationships, it is difficult to decide whether automixis is meiotic (reductive separation of centromeres) or mitotic (equational separation). Since a diploid *homozygote* has not been found as a product of automixis, the latter is preferred (see figure 3 for some of the postulated expectations). The term "somatic segregation" has been employed for analogous situations, but the issue here is not whether segregation occurs in a germinal or a somatic cell (whatever this would mean in the present context) but the underlying chromosome mechanics (STERN 1936).

The relationship of reduction to automixis is obscure. They may be concurrent, or as in *Aspergillus nidulans* (PONTECORVO, GLOOR, and FORBES 1954) the two processes may be independent, reduced crossover haploids being derived from syngenotes that

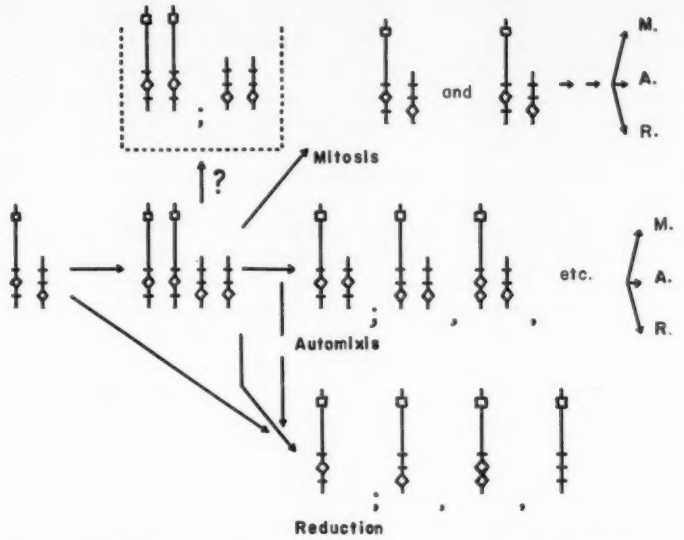

FIGURE 3.—Diagrammatic representation of modes of cell division occurring in syngenotic clones M, mitosis; A, automixis; R, reduction.

had undergone prior automixis. On this notion, reduction would result directly from the loss, nonreplication or nondisjunction of the exogenote. The prevalence of the endogenotic class of segregant is consistent with this view, but with most other formulations as well. This question may require a detailed pedigree analysis of a segregating clone before a definite answer is possible. Attempts to induce segregation of heterogenotes, e.g., with UV (cf. effects on heterozygotes, LEDERBERG *et al.* 1951) have been unsuccessful.

In figure 3, the exogenote is depicted as synapsed with the homologous endogenote. Specific synapsis must occur, at least to account for automictic events, but this is only one of several possibilities. On the one hand, the exogenote might be firmly attached to the endogenote, e.g., at the *Lp* site, to form a short "branch"; on the other it might lie free in the cell during vegetative growth.

The stability of heterogenotes in vegetative growth can be explained by assuming either 1) a definite association, synapsis or attachment, of the two elements throughout the division cycle or 2) regular equational disjunction of an independent exogenote which would behave, in effect, as a separate chromosome. Automictic events would necessitate at least an occasional synapsis even on the second view.

Whether it is transient or persistent, synapsis is a crucial mid-step of the transduction process. Transduction might be more prevalent than is now recognized, in the sense that exogenotes are transferred, but unless they retain the capacity for specific synapsis, they may be unable to attain a functional relationship with the cell. For example, the failure of lambda generally to mediate transduction of loci

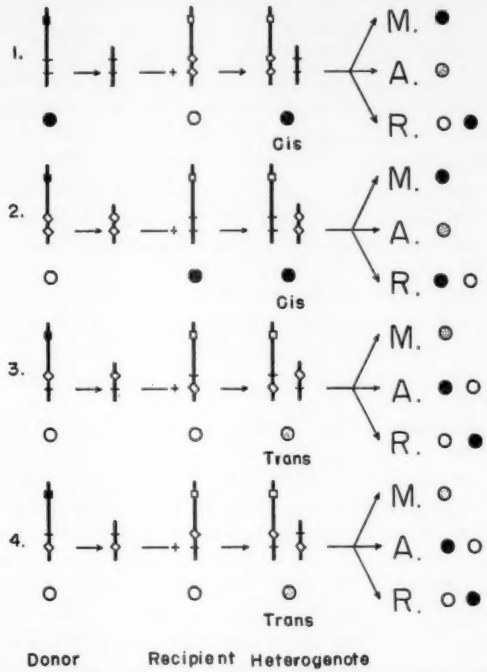

FIGURE 4.—The Gal_1^-, Gal_4^- interactions (position effect). The structural formulae, inferred from the origins of the clones and from subsequent segregational behavior, are shown diagrammatically. The phenotypes are: ●, galactose positive; dotted ○, galactose negative, papillating; ○, galactose negative.

remote from *Lp* might be explained in these terms, rather than by their failure to be enveloped in the lambda particle. To put the burden of specific synapsis on the *Lp* segment is a plausible extrapolation from the regularity with which lambda combines with an Lp^s cell to form a lysogenic Lp^+. However, other phages such as P1 do mediate generalized transduction in *E. coli* K-12. P1 and lambda might differ either in the quality of the nuclear fragments that are produced during viral growth (in regard to any critical step of transduction) or to the ability of the maturing phage coats to discriminate between prophage particles and other residues of the bacterial nucleus.

The uniqueness of each of the seven Gal^- markers that has been studied here parallels previous studies of the complexity of genetic loci. The uncovering of this complexity depends on the adequacy of recombination tests. In maize or Drosophila, 100,000 or a million tests would be considered exhaustive. The procedures described in this paper (HFT transduction and HFR crossing) could be extended to 10^9 or 10^{10} with comparable effort (compare crossing in maize, 10^5, LAUGHNAN 1955; LFT

transduction, 10^6, DEMEREC *et al.* 1955; recombination in phage, 10^6–10^8, BENZER 1955). The numerical comparisons should not be taken too seriously, as the most serious limitation is not the efficiency of the screening methods, but the interference from other factors, especially spontaneous mutation. Microorganisms have recently been the most prolific source of "pseudoallelism" but there is no reason to suppose that other organisms differ more profoundly than in limitations of technique.

Previous studies on bacteria have shown the prevalence of pseudoallelism and position effect for other loci in *E. coli* K-12. For example, heterozygotes of the *trans* type $Lac^-_{1a}Lac^+_{1b}/Lac^+_{1a}Lac^-_{1b}$ proved to be lactose-*negative*, while both single heterozygotes were lactose-positive (E. LEDERBERG 1952). Unfortunately, the techniques then available did not allow the positive identification of the *cis* heterozygote, $Lac^+_{1a}Lac^+_{1b}/Lac^-_{1a}Lac^-_{1b}$, which was assumed to be lactose-positive. In the *Gal* heterogenotes, the *cis* and *trans* arrangements have been compared directly (fig. 4), but they are yet to be studied in heterozygous diploids.

SUMMARY

The transduction of genetic material between cells of *E. coli* by phage lambda has been studied further. The material that could be transduced is limited to markers for the fermentation of galactose. Most of the transductional clones resulting carry a chromosomal fragment and are diploid for the genes which had been transduced. Such partially diploid clones, which have been named *heterogenotes*, segregate about once per thousand cell divisions, and give HFT^+ lysates after UV induction. With the exception of the gene combinations, $Gal^-_l Gal^+_i/Gal^+_l Gal^-_i$, heterogenotes of the form $+/-$, $+\ -/-\ +$, $-\ -/+\ +$, etc., were galactose positive, indicating that the + alleles are dominant. In the exceptional cases, *trans* combinations ($+\ -/-\ +$) had a negative, the *cis* ($-\ -/+\ +$) a positive phenotype, indicating a position effect between these loci. The heterogenotes undergo three modes of division: (1) mitosis, to propagate the original heterogenote; (2) reduction, to form haploid clones with markers of either the original recipient cell, transduced fragment, or recombinants of the two; (3) automixis, to form a new heterogenotic clone. This mode is analogous to somatic or mitotic crossing over.

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HETEROCARYONS BETWEEN STRAINS OF NEUROSPORA CRASSA WITH DIFFERENT CYTOPLASMS

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Received May 4, 1956

MITCHELL and MITCHELL (1952) and MITCHELL, MITCHELL and TISSIERES (1953) have shown that two strains of *Neurospora crassa*, *poky* and *mi 3*, of independent origin, differ from wild type and from each other in growth rate and cytochrome content. The differences are inherited maternally and thus appear to be associated with the cytoplasm. The object of this work has been to show what type of cytoplasm results when heterocaryons are made between strains having *poky* and *mi 3* cytoplasms or between either and wild type. It was hoped to show in particular (1) whether *poky* and *mi 3* cytoplasms interact to give wild type, (2) whether intermediate states of cytoplasm are to be found, and (3) whether either mutant, *poky* or *mi 3*, is "dominant" over wild type in a way which would suggest infection.

MITCHELL *et al.* (1953) report growing mixed cultures of *poky* and *mi 3* strains and obtained from the mixture no strain with a growth rate faster than either; but there is no certainty that heterocaryon formation occurred in these cultures. In the present work, the components of all heterocaryons were marked with biochemical mutant nuclear genes, usually pantothenicless (5531) in one component and p-aminobenzoicless (1633) in the other, so that when the two strains were grown together on minimal medium the heterocaryon might be at an advantage. It will be seen that heterocaryons were in fact formed, that is mixture of nuclei occurred and there was opportunity for mixture of cytoplasms.

MATERIALS AND METHODS

The strains used in the heterocaryons to be described were derived from two strains, 5531A (*pan-in-wild cytoplasm*) and 1633A (*pab-in-wild*) (BEADLE and TATUM 1945). Sometimes the two original strains themselves were used. (Throughout, "*wild*" will be used to denote wild type cytoplasm, and "+" wild type in respect of nuclear genes.) 5531A and 1633A were selected for use because they were found to form a heterocaryon together very readily. Also, to the author's knowledge, no case of a suppressor of either gene had been found[2]: in some circumstances a single strain carrying such a suppressor might be mistaken for a heterocaryon. The marker genes were introduced into *poky* and *mi 3* strains, and reisolated in wild cytoplasm, by the crosses shown in table 1, which involved the following strains: (1) a *poky* strain, 2303-2a (+-*in-po*), obtained by MRS. M. B. MITCHELL from a cross of *po*-1720-1A (MITCHELL and MITCHELL 1952); (2) an *mi 3* strain, 2543-3a (+-*in-mi 3*) (MITCHELL *et al.* 1953); (3) a standard wild type, 2293-2a (+-*in-wild*).

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[2] The 5531A and 1633A cultures used in the present work and the *pan* and *pab* strains derived from them were all tested on minimal medium: there was no indication that any of them carried a suppressor.

TABLE 1

Derivation of strains used in heterocaryons

Cross: Maternal (protoperithecial) parent first	Progeny: Ascospore isolates used in heterocaryons
2303-2a (+-*in-po*) × 1633A (*pab-in-wild*)	*pab-in-po*-1 to -7
2543-3a (+-*in-mi 3*) × 1633A (*pab-in-wild*)	*pab-in-mi 3*-1 and -2
2293-2a (+-*in-wild*) × 1633A (*pab-in-wild*)	*pab-in-wild*-2
2303-2a (+-*in-po*) × 5531A (*pan-in-wild*)	*pan-in-po*-1
2543-3a (+-*in-mi 3*) × 5531A (*pan-in-wild*)	*pan-in-mi 3*-1
pan-in-mi 3-1 × 5531A (*pan-in-wild*)	*pan-in-mi 3*-2 to -6
2543-3a (+-*in-mi 3*) × 5531A (*pan-in-wild*)	*pan-in-mi 3*-7 to -9
5531A (*pan-in-wild*) × *pan-in-mi 3*-1	*pan-in-wild*-2 and -3

Ascospore isolates from these crosses were tested as to their ability to form heterocaryons with a tester strain, in the case of the first crosses examined with one of the two original strains 5531A or 1633A, and the few isolates enumerated in table 1 were selected for further use. The majority, often more than 90%, were discarded because they were not found to form heterocaryons readily with the tester strain, that is, when the growth of a strain and the tester together on minimal medium was less than that of either on supplemented medium. For later crosses, tester strains which themselves formed a heterocaryon readily with one of the original strains were used. It was usually found that where one of two strains formed a heterocaryon readily with 5531A and the other with 1633A they also did so together. It was possible therefore to use isolates in heterocaryons with several strains. It was presumably a result of using 5531A and 1633A as the original tester strains, that most heterocaryons examined were between strains of mating type A.

Heterocaryons were made on minimal slants. Unless otherwise stated, the two components were inoculated on to the same point in the middle of a slant, one on top of the other, using approximately equal dry inocula (mycelium and conidia) from young cultures. These first slant cultures will be referred to throughout as *heterocaryon cultures*. In many cases they were tested as to their cytoplasmic properties by growth in flasks in minimal medium, as described by MITCHELL *et al.* (1953). Pads of mycelium resulting from four days' growth in flasks were examined for cytochrome content by observation with a hand spectroscope, and their dry weight was determined as a measure of the growth rate of the cultures. However it was not known to what extent any particular heterocaryon culture, or the mycelium grown from it in flasks, was in fact heterocaryotic. It was possible that it might consist of a mixture of the heterocaryon and its separate components, or even of the components alone supporting each other through the medium. In many cases therefore a transfer was made from the heterocaryon culture to a minimal plate from which a few single hyphal tips were isolated. Each one was transferred to a slant of minimal medium. The resulting cultures will be referred to as *hyphal tip cultures*. They were tested by growth in flasks in the same way as for the heterocaryon cultures. It could be assumed that the hyphal tips which grew had been heterocaryotic, and this was always confirmed by the recovery of both marker genes in those cases where single hyphal

TABLE 2

Control results

Dry weights from 4 days' growth in flasks of components of heterocaryons (marked with pab *or* pan) *on supplemented medium*

	wild (22 observations)	*poky* (35 observations)	*mi 3* (32 observations)
lowest	79 mg	2 mg	7 mg
highest	114 mg	22 mg	46 mg
average	87 mg	11 mg	30 mg

tip cultures were crossed to wild type (+-*in-wild*). Most attention will be given, therefore, to the cytoplasmic properties of the hyphal tip cultures.

An indication should be given of how much information was obtained from the tests of cultures by growth in flasks. Pads of *poky* mycelium have about 15 times, and of *mi 3* about 5 times, the concentration of cytochrome *c* in wild type pads (MITCHELL *et al.* 1953). With the hand spectroscope used in these experiments, it was possible to distinguish readily, on the basis of accumulation of cytochrome *c*, between *poky* and *wild* mycelium on the one hand and between *mi 3* and *wild* on the other. But it was not possible to distinguish with certainty between *poky* and *mi 3*, for which the difference in concentration of cytochrome *c* was offset by a difference in size of the pads. Single pads were examined. Also, with this hand spectroscope, it was not possible to detect the cytochrome *b* absorption band which distinguishes *wild* and *mi 3* mycelium from *poky*.

When heterocaryon or hyphal tip cultures were tested in flasks they were compared with their component strains grown in flasks of supplemented medium at the same time. The growth rates of these controls are summarized in table 2. It is clear that on the basis of growth rate, as well as cytochrome content, it is easier to distinguish between *poky* and *wild* and between *mi 3* and *wild* than between *poky* and *mi 3* for which the range of dry weights overlaps. An intermediate state of cytoplasm might most readily be detected in heterocaryons between *poky* and *wild* strains. It is satisfactory that the control growth rates agree with results previously obtained for different isolates of *poky* and *mi 3* strains not carrying biochemical marker nuclear genes, e.g. the samples quoted by MITCHELL and MITCHELL (1956). Except that perhaps *pab-in-mi 3* isolates grew rather slowly, there was no indication of interaction between the *pab* and *pan* genes and the *poky* and *mi 3* cytoplasms.

RESULTS

Heterocaryons between strains having wild and poky cytoplasms

Table 3 shows the results obtained for heterocaryons between three *pan-in-wild* strains (5531A and two reisolates of it) and five different isolates of *pab-in-poky*.

Ten replicates of one of these heterocaryons, *pan-in-wild*-3 with *pab-in-po*-4, were made, using as far as possible the same technique. The growth of the ten was uniform as heterocaryon cultures and much alike when these were transferred to minimal plates. Two replicates were selected as showing most difference from each other in

their growth on plates: Replicate 4, thought to be growing more slowly, and Replicate 5, thought to be growing faster. Single hyphal tips of these were isolated as recorded in the table.

The results in table 3 indicate that all these heterocaryons had wild type cytoplasm. All heterocaryon cultures and hyphal tip cultures examined had *wild* characteristics, and those heterocaryon cultures which were not tested gave every appearance of growing at wild type rate.

Two results not appearing in table 3 were thought at first to be exceptional. Heterocaryon cultures of 5531A (*pan-in-wild*) with *pab-in-po*-6 and 5531A with *pab-in-po*-7 appeared to be intermediate between *poky* and *wild*, in accumulation of cytochrome *c* and growth rate (32mg dry weight in both cases). One of these, 5531A with *pab-in-po*-6, was transferred to a minimal plate where its growth was slow and irregular so that hyphal tips were isolated with difficulty. Two out of five of these grew on minimal and were shown to resemble wild type in accumulation of cytochrome *c*, but to resemble *poky* in growth rate (10 mg and 18 mg dry weights). The type of growth on the plate suggested at once that this heterocaryon was not stable, and that this and not a *poky* cytoplasm might account for the low growth rate of the hyphal tip cultures. The cytoplasm may in fact have been *wild* as indicated by the cytochrome observations. It is considered likely that neither *pab-in-po*-6 nor *pab-in-po*-7 formed a stable heterocaryon with 5531A. It is of interest that both strains were found later to be mating type a.

TABLE 3

Heterocaryons between strains having wild *and* poky *cytoplasms* (pan-in-wild *with* pab-in-poky)

pan-in-wild component	*pab-in-po* component	Heterocaryon culture properties†	Hyphal tip cultures	
			Survival*	Properties†
5531A (*pan-in-wild*)	*pab-in-po*-1	wild	11/13	all wild
5531A (*pan-in-wild*)	*pab-in-po*-2	wild	6/9	all wild
5531A (*pan-in-wild*)	*pab-in-po*-3	wild	5/9	all wild
5531A (*pan-in-wild*)	*pab-in-po*-4	wild	— (Hyphal tips not isolated)	—
5531A (*pan-in-wild*)	*pab-in-po*-5	wild	—	—
pan-in-wild-2	*pab-in-po*-1	— (Culture not tested)	4/7	all wild
pan-in-wild-2	*pab-in-po*-2	—	4/7	all wild
pan-in-wild-2	*pab-in-po*-4	—	2/7	both wild
pan-in-wild-3	*pab-in-po*-1	—	5/7	all wild
pan-in-wild-3	*pab-in-po*-2	—	6/7	all wild
pan-in-wild-3	*pab-in-po*-4	—	1/7	wild
Replicates of *pan-in-wild*-3 with *pab-in-po*-4				
Replicate 4		—	5/7	all wild
Replicate 5		—	5/7	all wild

* Number surviving on minimal medium/number isolated.

† Cytoplasmic properties as indicated by observations of both cytochrome content and growth rate.

One heterocaryon was made between strains having *wild* and *poky* cytoplasms with the genes reversed, that is between the isolates *pab-in-wild*-2 and *pan-in-po*-1. The heterocaryon culture and six surviving hyphal tip cultures of seven isolated were tested in flasks, and all appeared *wild*.

Heterocaryons between strains having mi 3 and poky cytoplasms

Results for heterocaryons between four different isolates of *pan-in-mi 3* and five *pab-in-po* isolates are given in table 4. The *pab-in-po* isolates are those already mentioned as used in heterocaryons between *wild* and *poky* strains. As before, ten replicates were set up of one heterocaryon, *pan-in-mi 3*-7 with *pab-in-po*-3. One, replicate 6, was observed to grow less profusely than the others, both as a heterocaryon culture and when transferred to a minimal plate. Three replicates, including this one, but not necessarily including the fastest, were selected, and single hyphal tip cultures from them examined, as recorded in table 4. We have seen that *poky* and *mi 3* cultures could not be distinguished with complete certainty with the hand spectroscope,

TABLE 4

Heterocaryons between strains having mi 3 *and* poky *cytoplasms* (pan-in-mi 3 *with* pab-in-poky)

pan-in-mi 3 component	*pab-in-po* component	Heterocaryon culture properties‡	Hyphal tip cultures	
			Survival*	Properties‡
pan-in-mi 3-2	*pab-in-po*-1	mi 3	3/10	2 tested: both mi 3
pan-in-mi 3-2	*pab-in-po*-2	mi 3	8/18	4 tested: all mi 3
pan-in-mi 3-2	*pab-in-po*-3	mi 3	— (Hyphal tips not isolated)	—
pan-in-mi 3-2	*pab-in-po*-4	mi 3	—	—
pan-in-mi 3-2	*pab-in-po*-5	mi 3	—	—
pan-in-mi 3-7	*pab-in-po*-1	mi 3	7/10	all mi 3†
pan-in-mi 3-7	*pab-in-po*-2	mi 3	—	—
pan-in-mi 3-7	*pab-in-po*-3	Intermediate between mi 3 and wild	6/10	all mi 3†
pan-in-mi 3-8	*pab-in-po*-1	mi 3	—	—
pan-in-mi 3-8	*pab-in-po*-3	mi 3	—	—
pan-in-mi 3-9	*pab-in-po*-1	po	5/7	all po
pan-in-mi 3-9	*pab-in-po*-3	po	3/8	all po
Replicates of *pan-in-mi 3*-7 with *pab-in-po*-3				
Replicate 2		— (Culture not tested)	6/7	all mi 3
Replicate 6		—	6/7	all po
Replicate 10		—	3/7	all mi 3

* Number surviving on minimal medium/number isolated.

† Some of these hyphal tip cultures were used as protoperithecial parents in crosses with 2293-2a (+-*in-wild*). Both *pan* and *pab* mutants were recovered among ascospores from each cross examined.

‡ Cytoplasmic properties as indicated by observations of both cytochrome content and growth rate.

and that the ranges of their growth rates overlap. Thus the classification of cultures in this table is less satisfactory than that in tables 3 or 5.

The results in table 4 provide no evidence that *poky* and *mi 3* can interact to give wild type cytoplasm in a heterocaryon. No heterocaryon culture or hyphal tip culture was found to grow faster than its faster (*mi 3*) component. A single exception was the original heterocaryon culture of *pan-in-mi 3*-7 with *pab-in-po*-3, which gave a dry weight of 62mg, and its accumulation of cytochrome *c* agreed with this in suggesting a condition intermediate between *mi 3* and *wild*. But hyphal tip cultures derived from this heterocaryon culture and those from the replicates of it which were examined did not behave similarly. One possible explanation of the result is that a suppressor of *poky* (such as that described by MITCHELL and MITCHELL 1956), or of *mi 3*, was present in the heterocaryon culture, perhaps only during its growth in the flask.

While most of the cultures in table 4 were classified as *mi 3*, the heterocaryon and hyphal tip cultures from the heterocaryons involving *pan-in-mi 3*-9 consistently gave growth rates typical of *poky* cultures, though only limited support for this classification could be obtained spectroscopically. These two heterocaryons, like two between *poky* and *wild* strains discussed earlier, proved to be unusual in being between strains of opposite mating type—*pan-in-mi 3*-9 is a; perithecia and ascospores formed in both heterocaryon and hyphal tip cultures. However there was evidence, for example from the vigour of their growth on plates, that these were stable heterocaryons, unlike the earlier examples. Even so the slow growth rates obtained may possibly have been associated with the difference in mating type. GROSS (1952) and GARNJOBST (1953) have noted slow growth rates of heterocaryons between opposite mating types, though GARNJOBST recorded exceptions. Difference of mating type cannot be the explanation of the *poky* result for replicate 6 of *pan-in-mi 3*-7 with *pab-in-po*-3. That heterocaryons between *poky* and *mi 3* strains occasionally have *poky* cytoplasm, not *mi 3* cytoplasm, seems more likely in the light of the results to be discussed for heterocaryons between *mi 3* and *wild* strains.

Some of the cultures classified as *mi 3* in table 4 should perhaps have been entered as intermediate between *poky* and *mi 3* though tending towards *mi 3*. Among all the *mi 3* cultures, growth rates for hyphal tip cultures from the same heterocaryon culture agreed well within themselves; but sets of growth rates for hyphal tip cultures from different heterocaryon cultures sometimes differed significantly. For example, the following dry weights were obtained for hyphal tip cultures of *pan-in-mi 3*-7 with *pab-in-po*-1: 39mg; 33mg; 39mg; 33mg; 29mg; 30mg; 27mg, and the following for hyphal tip cultures from the original heterocaryon culture of *pan-in-mi 3*-7 with *pab-in-po*-3: 26mg; 20mg; 14mg; 25mg; 24mg; 30mg. The two sets of results were obtained at the same time using cultures of the same age. The second set is more suggestive of an intermediate condition than any other obtained. However, both sets fall well within the range of control results obtained for all *mi 3* components of heterocaryons, shown in table 2.

It should be noted that good agreement of the growth rates of hyphal tip cultures derived from the same heterocaryon culture, such as that illustrated by the data in the previous paragraph, was found throughout this work.

One heterocaryon was made between strains having *poky* and *mi 3* cytoplasms with

the genes reversed, between *pab-in-mi 3*-1 and *pan-in-po*-1. The heterocaryon culture only was tested, and was classified as *mi 3*.

Heterocaryons between strains having wild and mi 3 cytoplasms

Table 5 gives the results obtained for heterocaryons between 1633A (*pab-in-wild*) and four different isolates of *pan-in-mi 3*. The first results in the table, for heterocaryon cultures only, were among the earliest obtained for this combination of cytoplasms, and they suggested that the cytoplasm of the heterocaryons might be sometimes *mi 3* and sometimes *wild*—even differing for the same heterocaryon made on different occasions (in the case of 1633A with *pan-in-mi 3*-4). This heterocaryon was therefore further repeated, and made by four methods different from the usual one to show whether they influenced the results. The methods were: (I) a larger inoculum of the *mi 3* component than of the *wild* one was used; (II) a larger inoculum of the *wild* component was used; (III) the *pan-in-mi 3* component was first grown alone, on a slant containing a limiting amount of calcium pantothenate such that growth just covered the slant, and then the *pab-in-wild* strain was added; (IV) the *pab-in-wild* component was first grown alone, it grew sufficiently after several days on a minimal slant, and then the *pan-in-mi 3* strain was added. It will be seen that there was no indication, from the results for (I) and (II), that the cytoplasmic properties of the heterocaryon followed those of the component inoculated in larger amount, or, from

TABLE 5

Heterocaryons between strains having wild *and* mi 3 *cytoplasms* (pab-in-wild *with* pan-in-mi 3)

pab-in-wild component	*pan-in-mi 3* component	Heterocaryon culture Properties§	Hyphal tip cultures	
			Survival*	Properties§
1633A (*pab-in-wild*)	*pan-in-mi 3*-3	mi 3	— (Hyphal tips not isolated)	—
1633A (*pab-in-wild*)	*pan-in-mi 3*-5	wild	—	—
1633A (*pab-in-wild*)	*pan-in-mi 3*-6	wild	—	—
1633A (*pab-in-wild*)	*pan-in-mi 3*-4	mi 3	—	—
1633A (*pab-in-wild*)	*pan-in-mi 3*-4	wild	—	—
1633A (*pab-in-wild*)	*pan-in-mi 3*-4	Intermediate between mi 3 and wild	4/6	all mi 3‡
1633A (*pab-in-wild*)	*pan-in-mi 3*-4	I† wild	4/4	all mi 3‡
		II Intermediate between mi 3 and wild	4/4	all mi 3
		III wild	5/5	all wild‡
		IV wild	5/5	all wild‡

* Number surviving on minimal medium/number isolated.

† I–IV: Heterocaryons made by methods differing from the usual one (see text).

‡ Some of these hyphal tip cultures were used as protoperithecial parents in crosses with 2293-2a (+-*in-wild*). Both *pan* and *pab* mutants were recovered among ascospores from each cross examined.

§ Cytoplasmic properties as indicated by observations of both cytochrome content and growth rate.

the results for (III) and (IV), that they followed those of the component grown first. Results obtained for a second heterocaryon (1633A (*pab-in-wild*) with *pan-in-mi 3*-2), which was examined in the same way, presented a similar picture.

All these results suggested that a heterocaryon between strains having *mi 3* and *wild* cytoplasms might have the cytoplasmic properties of one or other component. Heterocaryon cultures tested appeared to be on different occasions *mi 3*, *wild* or intermediate. The sets of hyphal tip cultures derived from them were sometimes *mi 3* and sometimes *wild*. In spite of the results for some heterocaryon cultures, it is not clear that any case of a heterocaryon having an intermediate type of cytoplasm was obtained, since of the sets of hyphal tip cultures, which agreed well within themselves, none was evidently intermediate. The set of hyphal tip cultures from 1633A with *pan-in-mi 3*-4 made by method (IV) approached most nearly an intermediate condition. They gave growth rates which were somewhat low for wild strains (dry weights: 74 mg; 71 mg; 71 mg; 74 mg; 71 mg) and which were significantly different from growth rates obtained at the same time and under comparable conditions for hyphal tip cultures from method (III). Observations of cytochrome agreed with the classification of both sets of hyphal tip cultures as *wild*. The heterocaryon cultures classified as intermediate may be explained by supposing them to have been mixtures perhaps of an *mi 3* heterocaryon and its wild component (1633A (*pab-in-wild*) grows a very little on minimal medium). The case of a heterocaryon culture appearing *wild* and the hyphal tip cultures derived from it *mi 3* may require a similar explanation.

For the heterocaryon 1633A (*pab-in-wild*) with *pan-in-mi 3*-4 (as shown in the table), and for 1633A (*pab-in-wild*) with *pan-in-mi 3*-2, marker genes were recovered from crosses of both *mi 3* and *wild* hyphal tip cultures to wild type. Preliminary counts of *pab* and *pan* mutants at ascospore germination gave no indication that the cytoplasmic properties of the cultures were correlated with their nuclear ratios.

Six heterocaryons were made between strains having *mi 3* and *wild* cytoplasms with the *pab* and *pan* genes reversed, using the strains 5531A (*pan-in-wild*), *pan-in-wild*-2 and *pan-in-wild*-3 with *pab-in-mi 3*-1 and *pab-in-mi 3*-2. Only heterocaryon cultures were tested and all were classified as *wild*.

Heterocaryons were also made between the following strains obtained by MRS. M. B. MITCHELL, in which the nuclear markers were C141 (histidineless) and 34556 (pantothenicless): *hist-in-wild* with *pan-in-mi 3*, and *pan-in-wild* with *hist-in-mi 3*. (The mutant *pan* (34556) has not been shown to be different from *pan* (5531)). Ten replicates were made of both of these heterocaryons in the way already described for a heterocaryon between *poky* and *wild* strains. For both, the replicates thought to be the fastest and slowest from their growth on minimal plates were selected, and hyphal tip cultures from them were examined. The hyphal tip cultures from both replicates of *hist-in-wild* with *pan-in-mi 3* were classified as *mi 3*, and those from both replicates of *pan-in-wild* with *hist-in-mi 3* as *wild*.

DISCUSSION

The results obtained for heterocaryons between strains having *poky* and *mi 3* cytoplasms indicate that such strains do not interact to give wild type mould or a strain faster than *mi 3*.

No stable intermediate state of cytoplasm has been shown to occur. For hetero-

caryons between *poky* and *wild* strains, where this might most easily have been detected, no set of hyphal tip cultures was found which could not confidently be classed as *wild*. For both of the other combinations of cytoplasms, the set of hyphal tip cultures which came nearest to suggesting an intermediate state of cytoplasm has been described, but the properties of each set were sufficiently typical of one of the component cytoplasms to be regarded as indicating only a slight variation in the manifestation of it.

Thus all cultures which were known to have been heterocaryotic (derived from single hyphal tips) were shown to have the cytoplasmic properties of one of their components.[3] In the case of heterocaryons between *mi 3* and *wild* strains different sets of hyphal tip cultures had the properties sometimes of one and sometimes of the other component, even differing for a single heterocaryon made on different occasions from the same strains. The *mi 3* with *wild* heterocaryons were unlike those for *poky* with *wild* and *poky* with *mi 3* in having, in most cases, the pantothenicless gene in the slower component; for all the combinations of cytoplasms more information about heterocaryons with the genes reversed and about heterocaryons with different marker genes is very desirable. However, it may be that heterocaryons between the other combinations of cytoplasms, as well as *mi 3* with *wild*, may take two alternative forms, which each have the cytoplasmic properties of one component, and which perhaps arise by exchange of nuclei without exchange of cytoplasms, or by segregation of the cytoplasms from a temporary mixture. One set of hyphal tip cultures, at least, suggests that there may be a *poky* form, as well as an *mi 3* form, of *poky* with *mi 3* heterocaryons. Failure to obtain *poky* hyphal tip cultures from *poky* with *wild* heterocaryons may have been a consequence of the large difference between the growth rates associated with *poky* and *wild* cytoplasms, that is, a consequence of the selective advantage of the *wild* form of heterocaryon. If so, it is not evident why, for heterocaryons between the other combinations of cytoplasms where the growth rates of the components differed less, mixtures of hyphal tip cultures of the two possible sorts were not obtained from the same heterocaryon culture, though hyphal tips of any one set were often derived from inoculations on to more than one plate. If there are two forms of heterocaryon for each combination of cytoplasms, the form assumed by a particular culture may be decided at an early stage, perhaps when the heterocaryon is first formed, by some mechanism of which there is no indication.

It is evident from the consistent finding that *poky* with *wild* heterocaryons have the characteristics of strains with *wild* cytoplasm, that the *poky* condition is not "dominant" over *wild* in the way which might be expected if *poky* was caused by, and able to infect wild cytoplasm with, a virus. As will be clear from the previous paragraph, it does not necessarily follow that *wild* is "dominant" over *poky*, or hence that *poky* cytoplasm has a deficiency which can be repaired by *wild*. Also, the results do not indicate that either component is "dominant" in *mi 3* with *wild* heterocaryons.

SUMMARY

Heterocaryons have been made between strains of *Neurospora crassa* having *poky*, *mi 3* and *wild type* cytoplasms, in the three possible combinations. The strains were

[3] The method of selecting the strains used in heterocaryons means that the possibility of a heterocaryon with a growth rate slower than that of its slower component has not been examined.

marked with biochemical mutant nuclear genes and the heterocaryons made on minimal medium. Single hyphal tips were isolated and were confirmed as having been heterocaryotic, in all cases tested, by recovery of the marker genes of both components of the heterocaryon.

The cytoplasmic properties of the heterocaryons, as indicated by tests of the single hyphal tip cultures, were: for a number of heterocaryons between strains having *poky* and *wild type* cytoplasms respectively, consistently *wild type;* for heterocaryons between *poky* and *mi 3* strains, usually *mi 3* but occasionally *poky;* for heterocaryons between *mi 3* and *wild type* strains, sometimes *mi 3* and sometimes *wild type* cytoplasm. Thus:

(1) There was no evidence that strains having *poky* and *mi 3* cytoplasms can interact in a heterocaryon to give wild type mould or a strain faster than either.

(2) No intermediate state of cytoplasm was found.

(3) *Poky* and *mi 3* were not shown to be "dominant" over *wild type* in the way which might be expected if either condition was caused by an infective agent.

ACKNOWLEDGMENTS

This investigation was begun in association with MRS. M. B. MITCHELL and DR. H. K. MITCHELL, and I wish to express my indebtedness to them for their invaluable help and encouragement throughout its course. I also wish to thank DR. G. W. BEADLE and DR. G. H. BEALE for their advice, and PROFESSOR G. PONTECORVO for his interest in the work and for generously allowing me the hospitality of his department.

This work was undertaken during tenure of a Gosney Post-doctoral Research Fellowship in Biology at the California Institute of Technology, and continued with a grant from the Medical Research Council of Great Britain.

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